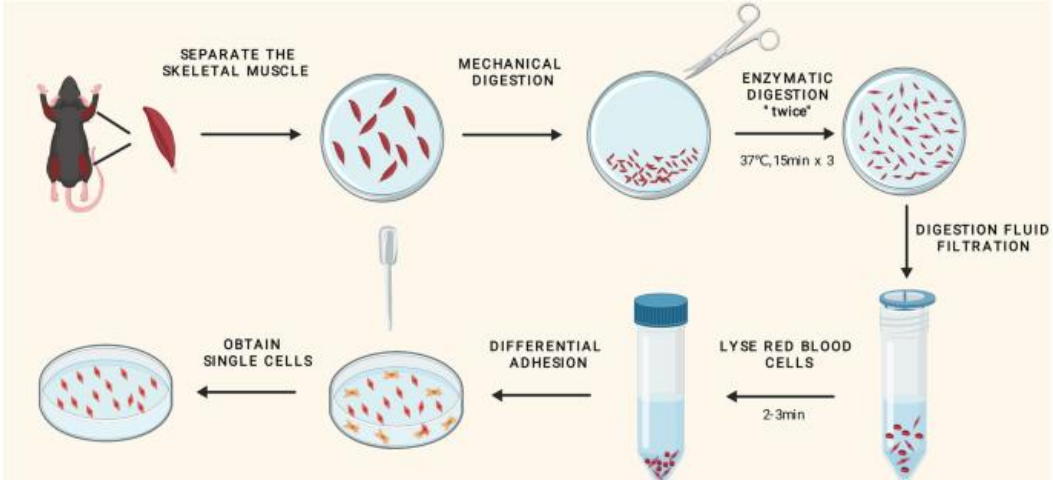
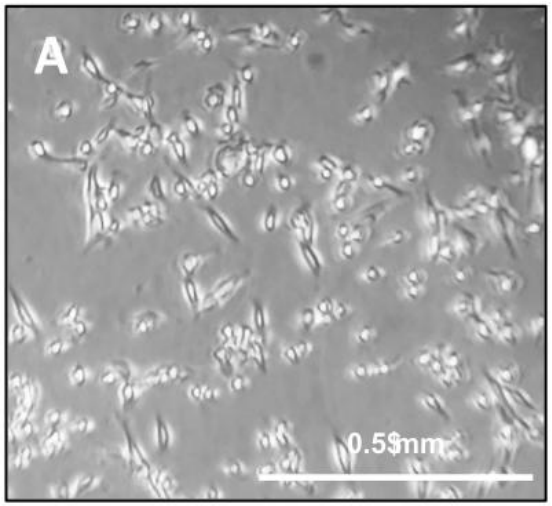
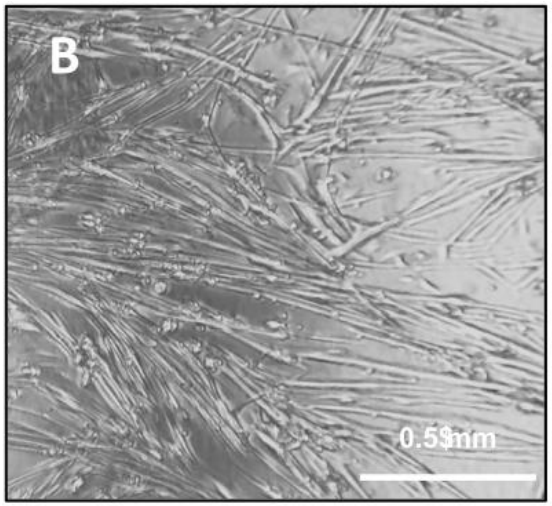
Isolation and Differentiation of Primary Myoblasts from Mouse Skeletal Muscle Explants

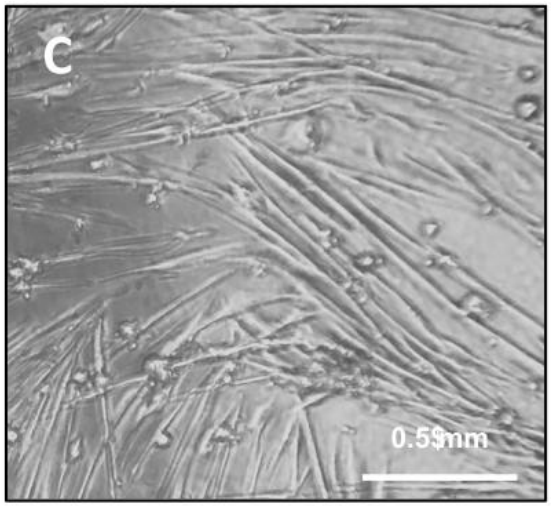




Day'1



Day'5



Day'6

Myotubes formed after 5 days of induction in differentiation medium (DM);

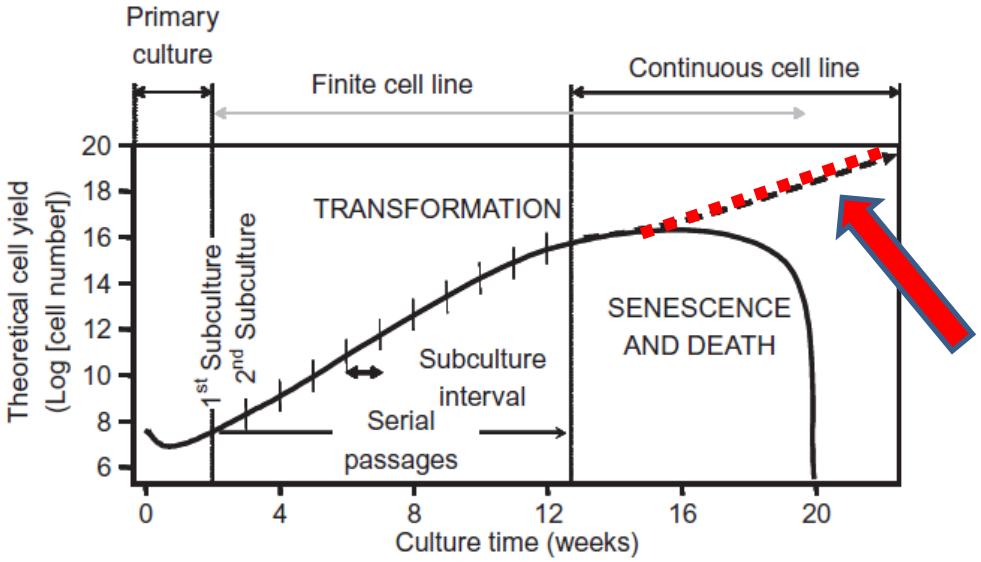
https://www.stemcell.com/how-to-prepare-a-single-cell-suspension-from-mouse-spleen.html

https://www.youtube.com/watch?v=gePfThd2Hro

Replicative senescence

in animal cells growing in vitro was first discovered by Leonard Hayflick.

He found that primary human diploid fibroblast cell lines **ceased to proliferate after an extended number of serial passages** (Hayflick, 1965).



- This phenomenon occurs **independently** of the presence of metabolites appropriate for growth.

- A major causal feature of replicative senescence is **telomere erosion**, a process in which the telomeres gradually shorten with increasing cellular divisions.

Continuous cell lines arise from single cells in which **spontaneous or induced mutations** have abrogated the genetic program of senescence.

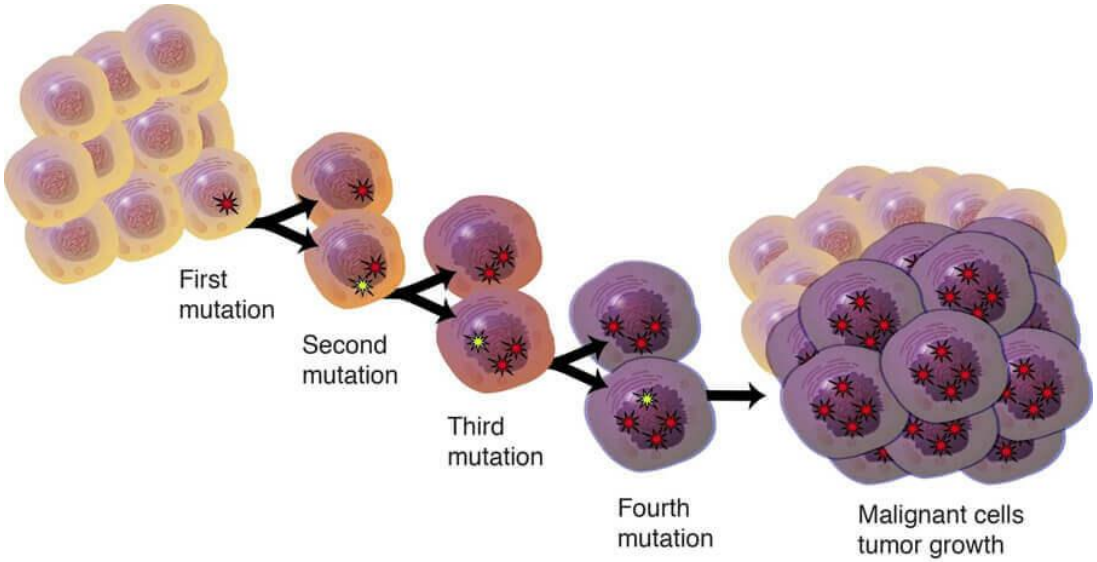
They are therefore called **immortal**: they proliferate continuously in the presence of the appropriate metabolites. Many continuous cell lines have been obtained from tumor tissues (e.g. HeLa).

The transformation process makes a cell line immortal.

It begins with the acquisition by the cell line of the **ability to proliferate indefinitely** (immortalization);

it requires a certain number of mutations in different genes.

When it occurs in culture (even spontaneously**), foci of transformation are observed** under the microscope, that is, masses of cells similar to tumor cells, which are no longer affected by contact inhibition.



Alcune linee cellulari usate comunemente in laboratorio

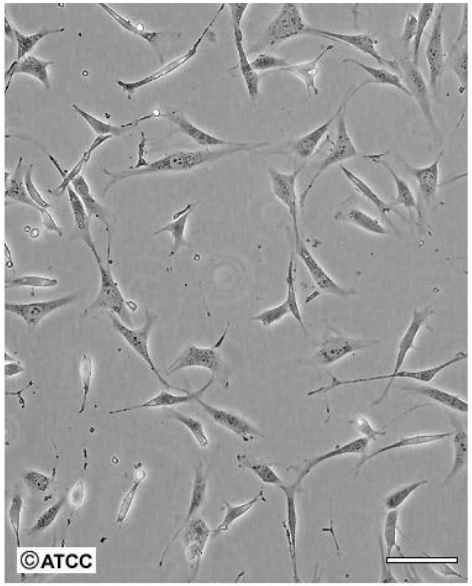
3T3	fibroblasto (topo)
BHK21	fibroblasto (hamster)
MDCK	cellula epiteliale (cane)
HeLa	cellula epiteliale (uomo)
PtK1	cellula epiteliale (ratto canguro)
L6	mioblasto (ratto)
PC12	cellula cromaffine (ratto)
SP2	plasmacellule (topo)
COS	rene (scimmia)
293	rene (uomo) trasformata con Adenovirus
CHO	ovaio (hamster)
DT40	cellula di linfoma per ricombinazione mirata efficiente (pollo)
R1	cellule staminali embrionali (topo)
E14.1	cellule staminali embrionali (topo)
H1, H9	cellule staminali embrionali (uomo)
S2	cellule similmacrofagiche (Drosophila)

Molte di queste linee cellulari sono derivate da tumori. Tutte sono capaci di replicazione indefinita in coltura ed esprimono almeno alcune delle caratteristiche peculiari della loro cellula di origine.
Le cellule BHK21, HeLa e SP2 sono capaci di crescere in modo efficiente anche in sospensione, le altre richiedono un substrato solido per crescere e moltiplicarsi.

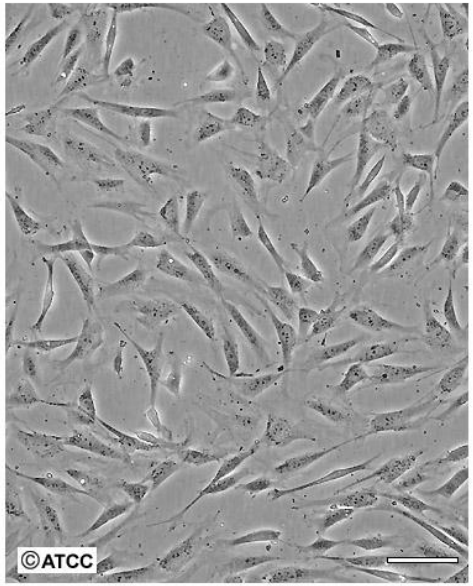
MOUSE 3T3 Fibroblast

3T3 cells come from a cell line established in **1962** by two scientists then at the Department of Pathology in the New York University School of Medicine,

ATCC Number: **CRL-1658**
Designation: **NIH/3T3**



Low Density Scale Bar = 100µm

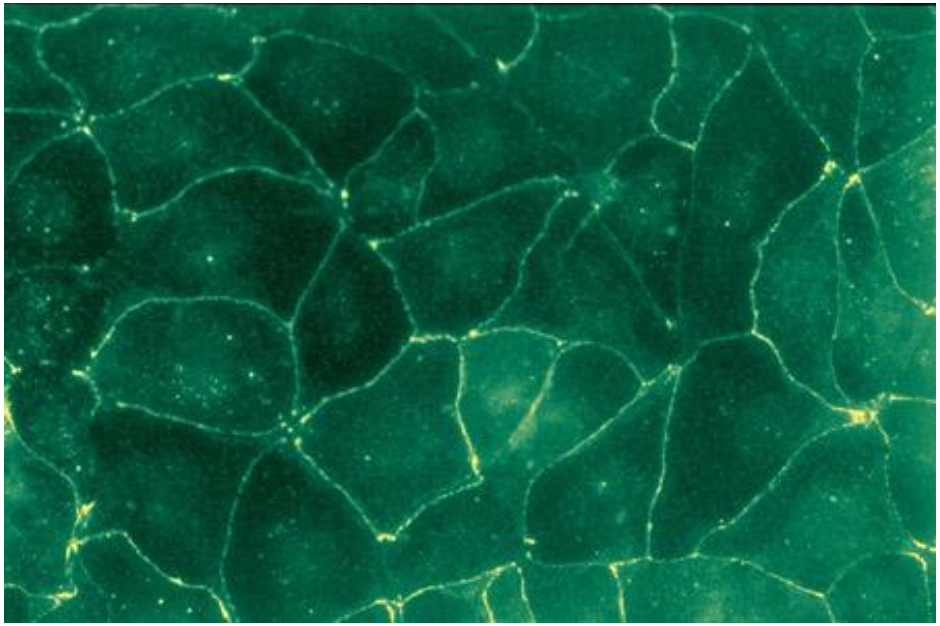


High Density Scale Bar = 100µm

Canine MDCK (epitelial)

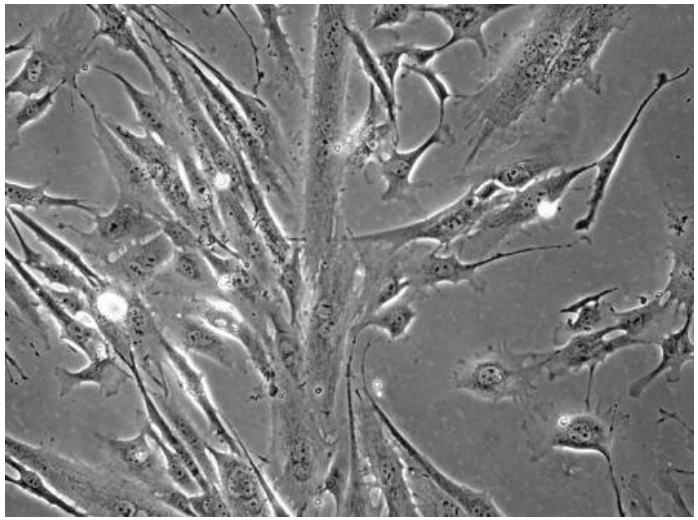
(Madin-Darby Canine Kidney)

Derived from a **kidney** of an apparently normal adult female cocker spaniel in September **1958**



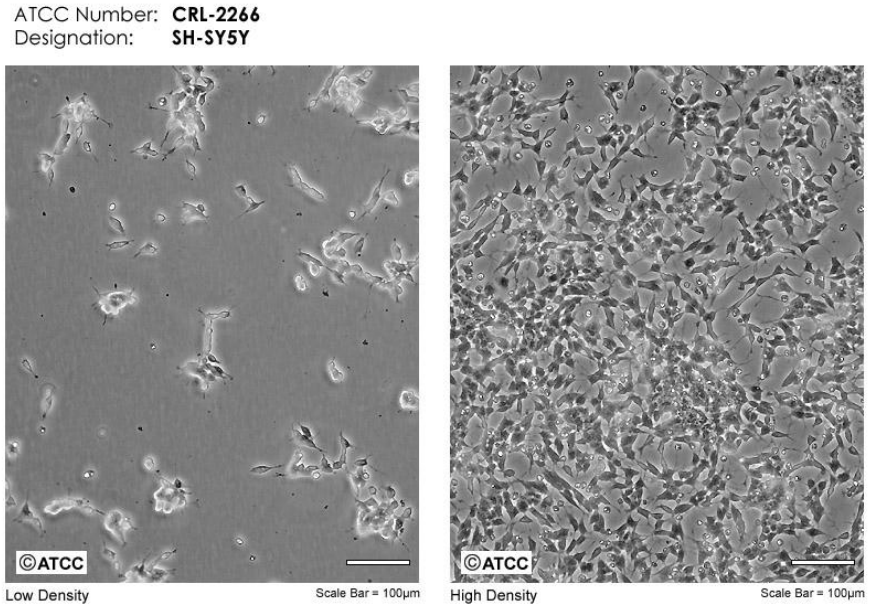
RAT L6

Mioblast

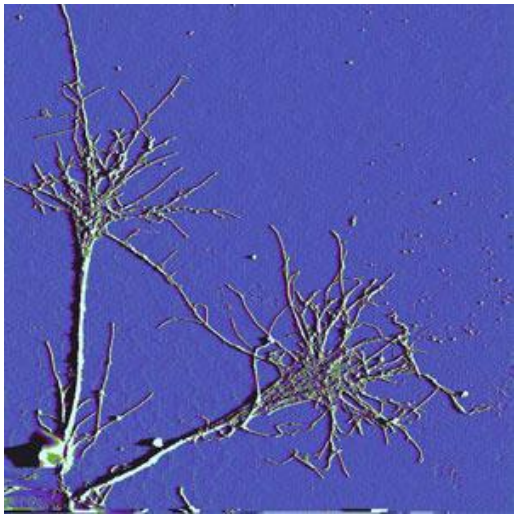


SH SY5Y Neuronal

The cell line **SH-SY5Y** is a thrice-cloned **neuroblastoma,** originally from SK-N-SH and first reported in 1978.



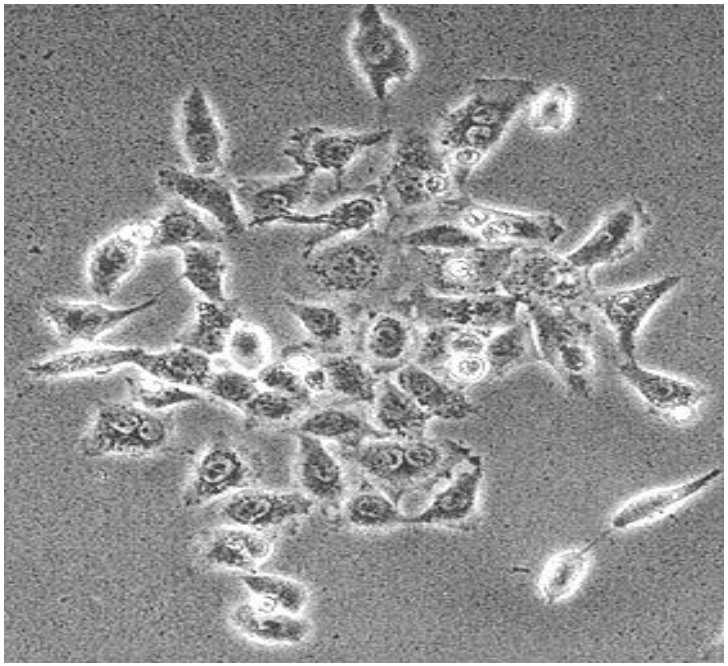
+ retinoic acid

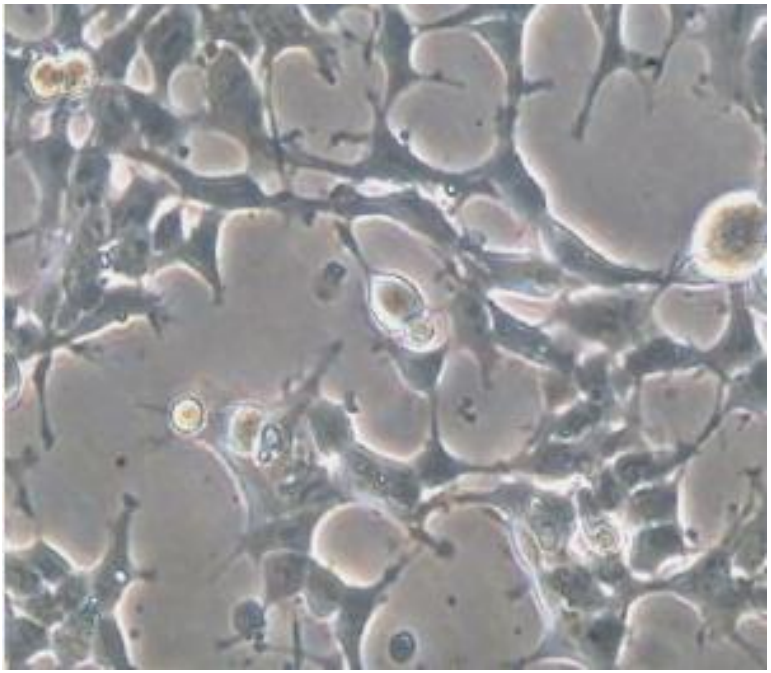


HEK 293

Origins of the HEK293 Cell Line

HEK293 is a cell line derived in 1973 from a spontaneously miscarried or aborted female fetus or **H**uman **E**mbryonic K**i**dney





https://www.hek293.com/

HEALTH DECEMBER 21, 2020 / 1:44 PM / UPDATED 2 MONTHS AGO

Vatican permits use of COVID-19 vaccines made using aborted fetal tissue

By Philip Pullella

2 MIN READ

VATICAN CITY (Reuters) - The Vatican told Roman Catholics on Monday that it was morally acceptable to use COVID-19 vaccines even if their production employed cell lines drawn from tissues of aborted foetuses.

Linee cellulari particolarmente utilizzate.

3T3 fibroblast (mouse)

BHK21 fibroblast (Syrian hamster)

MDCK epithelial cell (dog)

HeLa epithelial cell (human)

PtK1 epithelial cell (rat kangaroo)

L6 myoblast (rat)

PC12 chromaffin cell (derived from a rat pheochromocytoma)

COS fibroblast (monkey)

CHO ovary (chinese hamster)

HEK-293 generated by transformation of embryonic kidney cells (human)

Primary cells

closely represent the physiological state of a particular cell type in vivo, but they are susceptible to replicative senescence, so their value in the laboratory setting is limited.

Continous cell lines,

are not encumbered by replicative senescence, but, they often contain numerous genetic mutations, exhibit an unstable karyotype and have protein expression patterns that are not comparable with the cell type they are intended to represent.

	Primary Cells	Continuous
Mimic *in vivo* Tissue Phenotype	++++	+
Karyotypic Stability	Diploid	Aneuploid
Proliferative Capacity	+	+++
Supply	+	+++
Inter-Experimental Reproducibility	Low	Good
Cost	High	Low
Ease of Use	+	+++

G3: Genes|Genomes|Genetics Early Online,
published on March 11, 2013 as doi:10.1534g3.113.005777

The genomic and transcriptomic landscape of a HeLa cell line

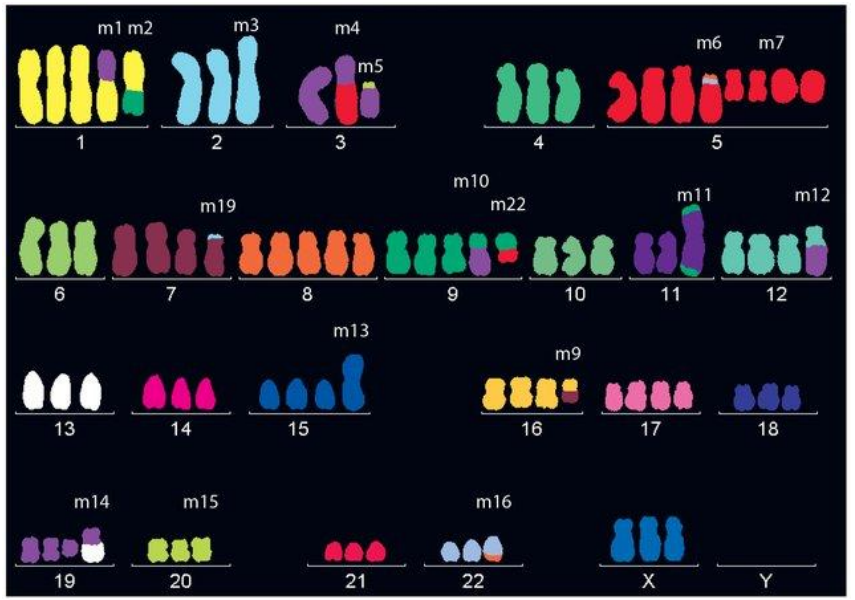
Jonathan Landry[1]*, Paul Theodor Pyl[1]*, Tobias Rausch[1], Thomas Zichner[1], Manu M. Tekkedil[1], Adrian M. Stütz[1], Anna Jauch[2], Raeka S. Aiyar[1], Gregoire Pau[1]†, Nicolas Delhomme[1]‡, Julien Gagneur[1#], Jan O. Korbel[1], Wolfgang Huber[1]§, Lars M. Steinmetz[1]§

Indeed, substantial chromosomal aberrations in the HeLa cell line have been revealed by cytogenetic methods

A combination of these techniques (CGH), (FISH), (SKY)] has been used to determine the karyotype of a CCL2 HeLa cell line
This cell line contained two subclonal populations, which were both hypertriploid (3n+), with

- **a variable total number of chromosomes (76−80)**

- a variable number of abnormal chromosomes (22−25) per cell



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Proliferative Capacity	+	+++
Supply	+	+++
Inter-Experimental Reproducibility	Low	Good
Cost	High	Low
Ease of Use	+	+++

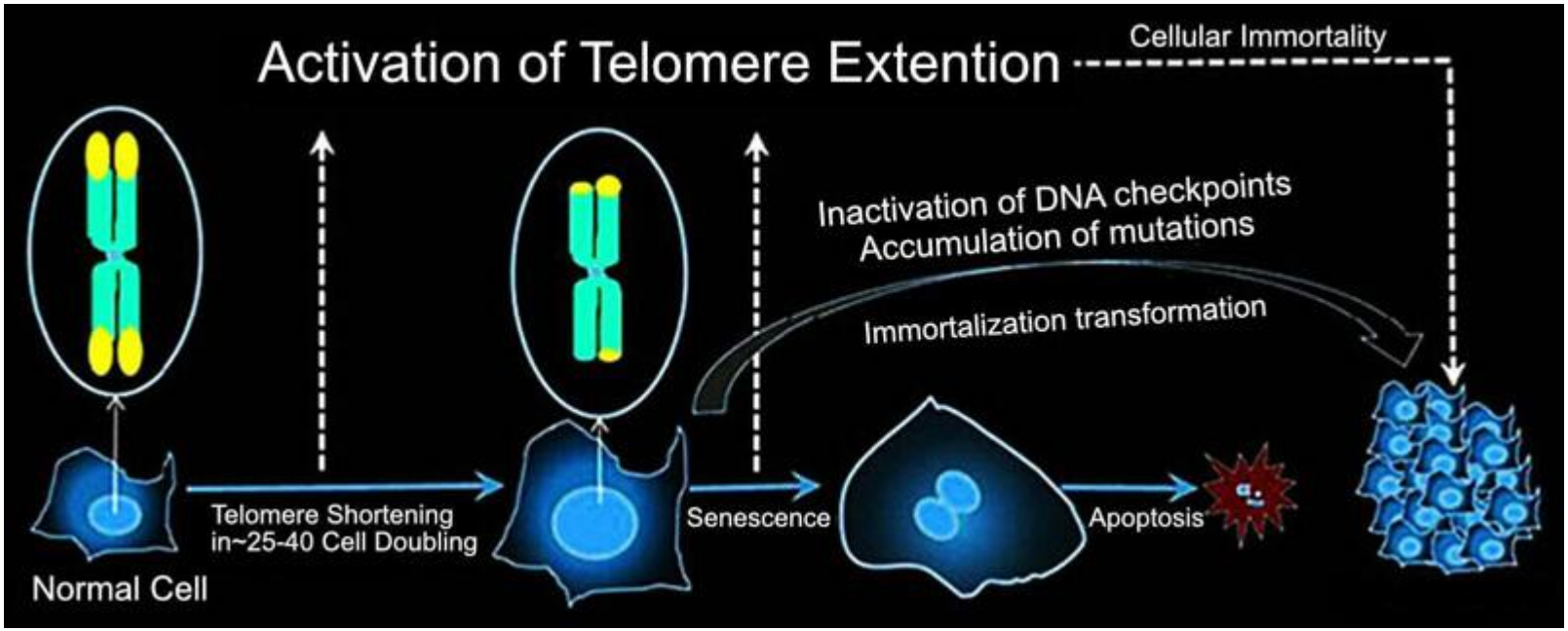
Strategies for Creating Immortal Cells

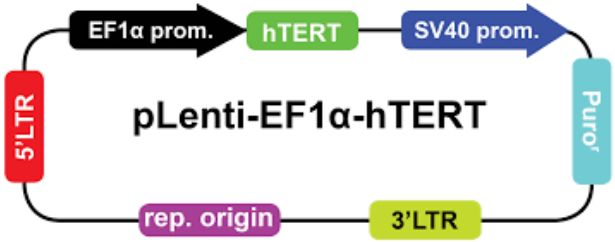
Several methods exist for immortalizing mammalian cells in culture conditions.

- One method is to use **viral genes**, **such as the simian virus 40 (SV40) T antigen,** to induce immortalization.

SV40 T antigen has been shown to be the simplest and most reliable agent for the immortalization of many different cell types and the mechanism of SV40 T antigen in cell immortalization is relatively well understood. The transforming activity of TAg is due in large part to its **perturbation of the retinoblastoma (pRb) and p53 tumor suppressor proteins**.

- The most recently discovered approach to cell immortalization is through the expression of **Telomerase Reverse Transcriptase protein** **(TERT),** particularly for cells that are most affected by telomere length, such as human cells

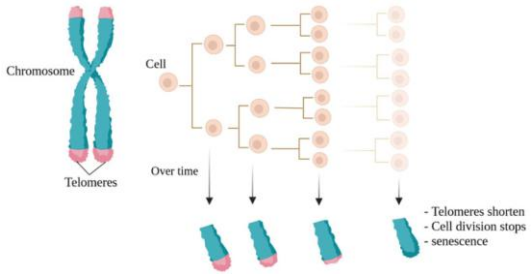




The enzyme **telomerase** can prevent the shortening of telomeres

the transfer of exogenous hTERT cDNA (encoding the catalytic subunit of human telomerase)

can be used to prevent telomere shortening, overcome telomere-controlled senescence, and immortalize primary human cells.



hTERT Immortalization of Primary Cells

Transfection of hTERT into human primary cells leads to **elongation and maintenance of the telomere ends of the chromosomes.**
In many instances, **forced expression of hTERT alone** enables the cells to repress replicative senescence and overcome the growth crisis, effectively leading to their immortalization.

In some cases, **more than one immortalization agent may be required** to successfully immortalize a particular cell type. For example primary cell lines may be immortalized using **a combination of hTERT with one or more of the following** genes encoding:

- viral (simian virus 40 (SV40) **large T antigen**
- human papilloma virus-16 (HPV-16) **E6/E7**)
- non-viral (Cdk-4 and Bmi-1) oncoproteins.

hTERT immortalized cells are **mostly diploid,** but may become pseudo-diploid especially at high passage number.

In many cases, when cells become pseudo-diploid they still retain most primary cell functions.

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Genetic Materials

Cell Immortalization Reagents

Immortalized cells are highly preferred over primary cells due to their ability to provide unlimited, consistent access to the same cell line. This eliminates the issues of batch-to-batch variability and the high costs associated with primary cell use.

As a pioneer in cell immortalization technology, **abm** offers:

- A comprehensive library of **immortalized cell lines** for human, mouse, and other key research model organisms.
- Industry-leading **immortalization reagents** for researchers who wish to immortalize their own primary cells.

Our selection features the widest range of reagents and the highest quality standards – areas where competitors simply can't compare. Whether you're seeking ready-to-use cell lines or advanced tools to immortalize your own, **abm** has you covered.

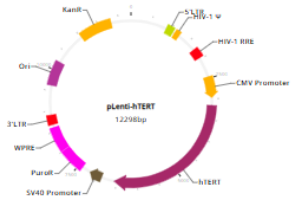
Recombinant SV40T Virus

SV40 T Antigen: Proven, Robust, and Versatile

Product Name	Cat. No.	Vector Map	Promoter	Marker	Price
Lentivirus					
Lenti-SV40T (Puro) Virus	LV613	View	CMV	Puro	€1,537.50
Lenti-SV40T (Neo) Virus	LV660	View	CMV	Neo	€1,537.50
Lenti-SV40T Virus	LV665	View	CMV		€1,537.50
Lenti-SV40Tt Virus	LV614	View	CMV	Puro	€1,537.50
Lenti-SV40 Virus	LV612	View	CMV		€1,537.50
Lenti-SV40T (tsA58 temp sensitive) (Puro) Virus	LV630	View	CMV	Puro	€1,537.50

https://www.abmgood.com/Cell-Immortalization.html

Lenti-hTERT (Puro) Virus, High Titer



Cat. No. LV615
Unit 2 x 100 µl
Price €1,537.50

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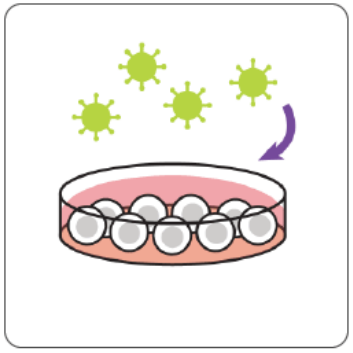
Specifications | Datasheet | Documents | FAQs | References | Reviews

Print

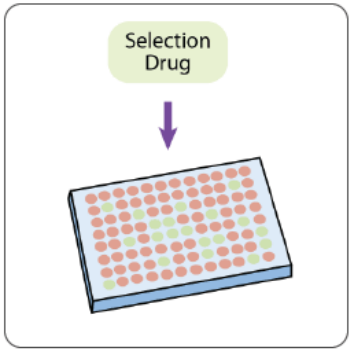
Cat. No.	LV615
Name	Lenti-hTERT (Puro) Virus, High Titer
Unit	2 x 100 µl
Unpacking and Storage Instructions	Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the viruses at -80°C in small aliquots to avoid repeated freeze-thaw cycles.
Description	High Titer (10^9 IU/ml) Recombinant Lentivirus expressing the hTERT gene.
Application	Cell immortalization.
Expression System Type	Lentivirus

abm's Cell Immortalization Workflow

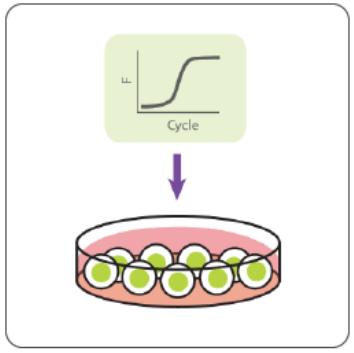
Viral transduction using immortalization agents on primary cells



Drug selection on successfully transduced cells



Clone selection and confirmation of immortalization via qRT-PCR of transgene



Cryopreservation of immortalized cells

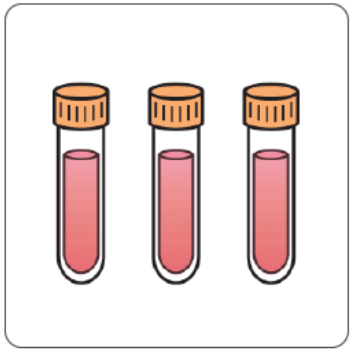
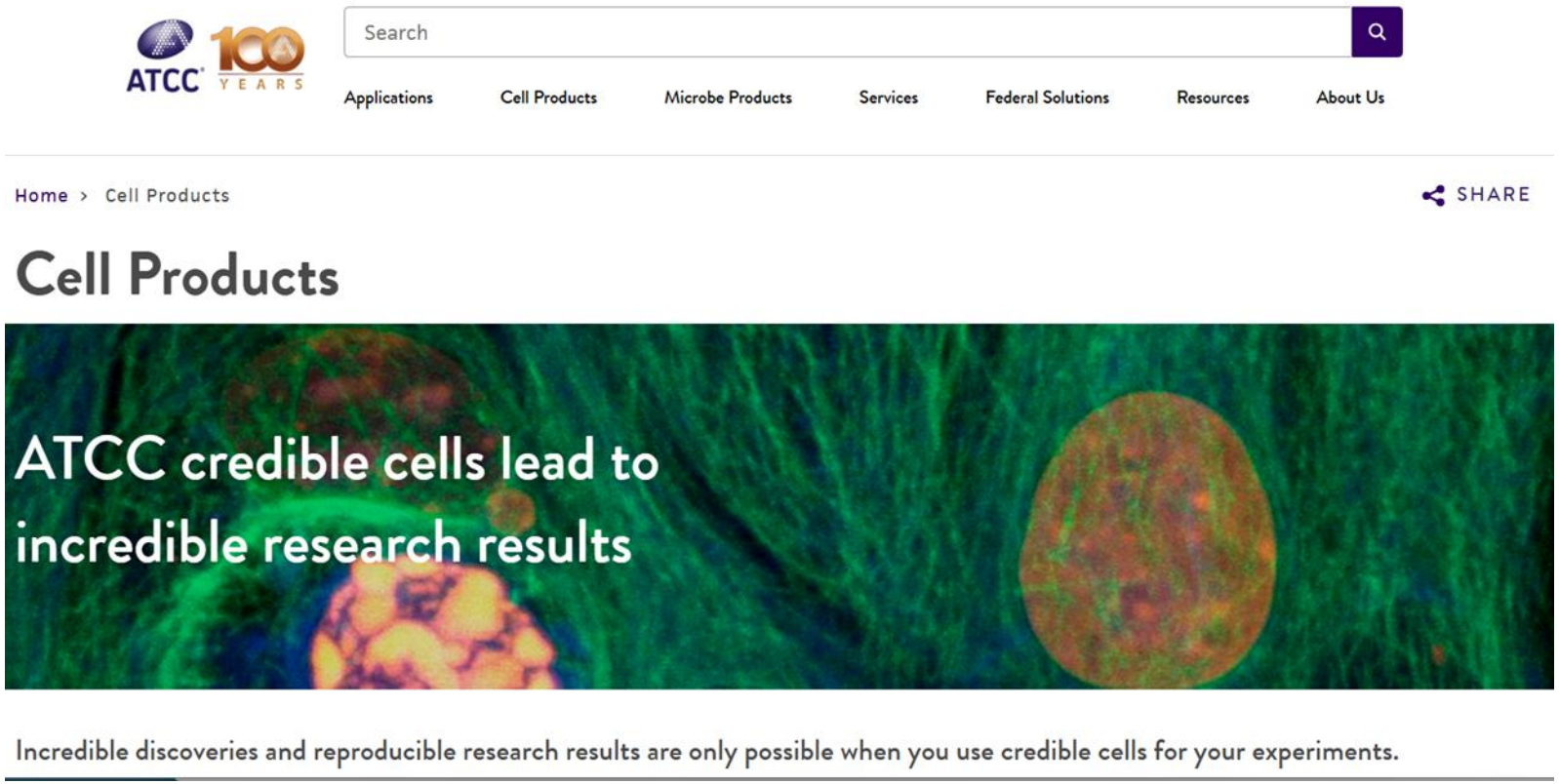


Table 1. Comparison Between hTERT Immortalized Cells, Primary Cells, Oncogene/Viral Immortalized Cells and Continuous Cell Lines

	Primary Cells	**hTERT-Immortaized**	**Onco, Viral-Immortalized**	**Continuous**
Mimic *in vivo* Tissue Phenotype	++++	+++	++	+
Karyotypic Stability	Diploid	Diploid/ Pseudodiploid	Pseudodiploid/ Aneuploid	Aneuploid
Proliferative Capacity	+	+++	+++	+++
Supply	+	+++	+++	+++
Inter-Experimental Reproducibility	Low	Good	Good	Good
Cost	High	Medium	Low	Low
Ease of Use	+	++	++	+++

WHERE CAN I FIND A CELL LINE OF INTEREST??



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- **acquisition**,
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- **development,**
- **distribution**

of **standard reference microorganisms, cell lines, and other materials**.

While maintaining traditional collection materials, ATCC develops high quality products, standards, and services to support scientific research and breakthroughs that improve the health of global populations.

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HELA

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CRM-CCL-2 BSL 2

96/100 Bioz Stars 7,283 Product Citations

Product format: Frozen
Product type: Certified reference material
Organism: *Homo sapiens*, human
Tissue: Uterus; Cervix
Disease: Adenocarcinoma
Cell type: epithelial cell

Quick View Compare

Price: **$968.00** ea

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HeLa

CCL-2 BSL 2

99/100 Bioz Stars 21,308 Product Citations

Product format: Frozen
Organism: *Homo sapiens*, human
Tissue: Uterus; Cervix
Disease: Adenocarcinoma
Cell type: epithelial cell

Quick View Compare

Explore our data

Price: **$577.00** ea

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HeLa S3

CCL-2.2 BSL 2

96/100 Bioz Stars 1,666 Product Citations

Product format: Frozen
Organism: *Homo sapiens*, human
Tissue: Uterus; Cervix

Price: **$577.00** ea

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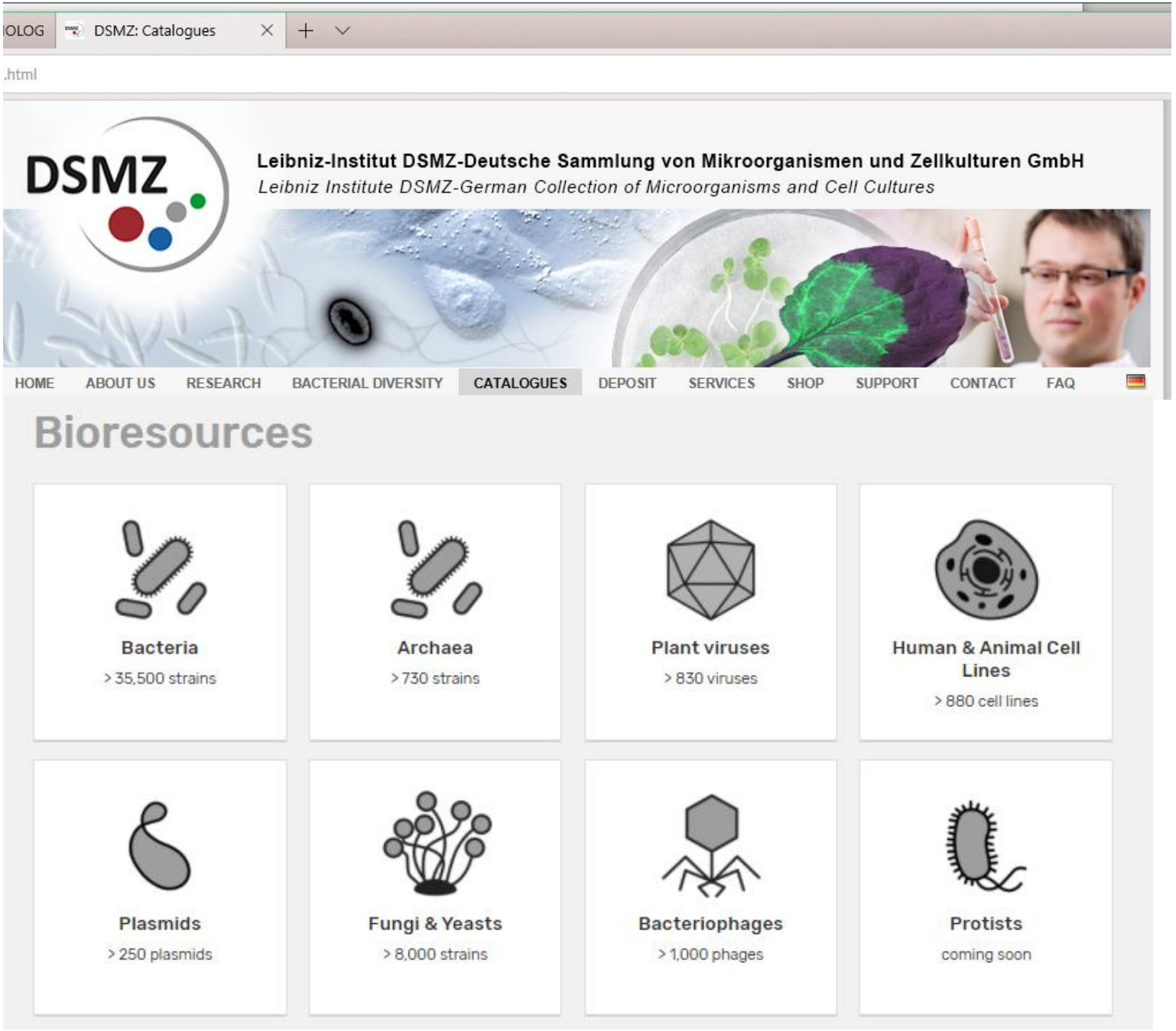
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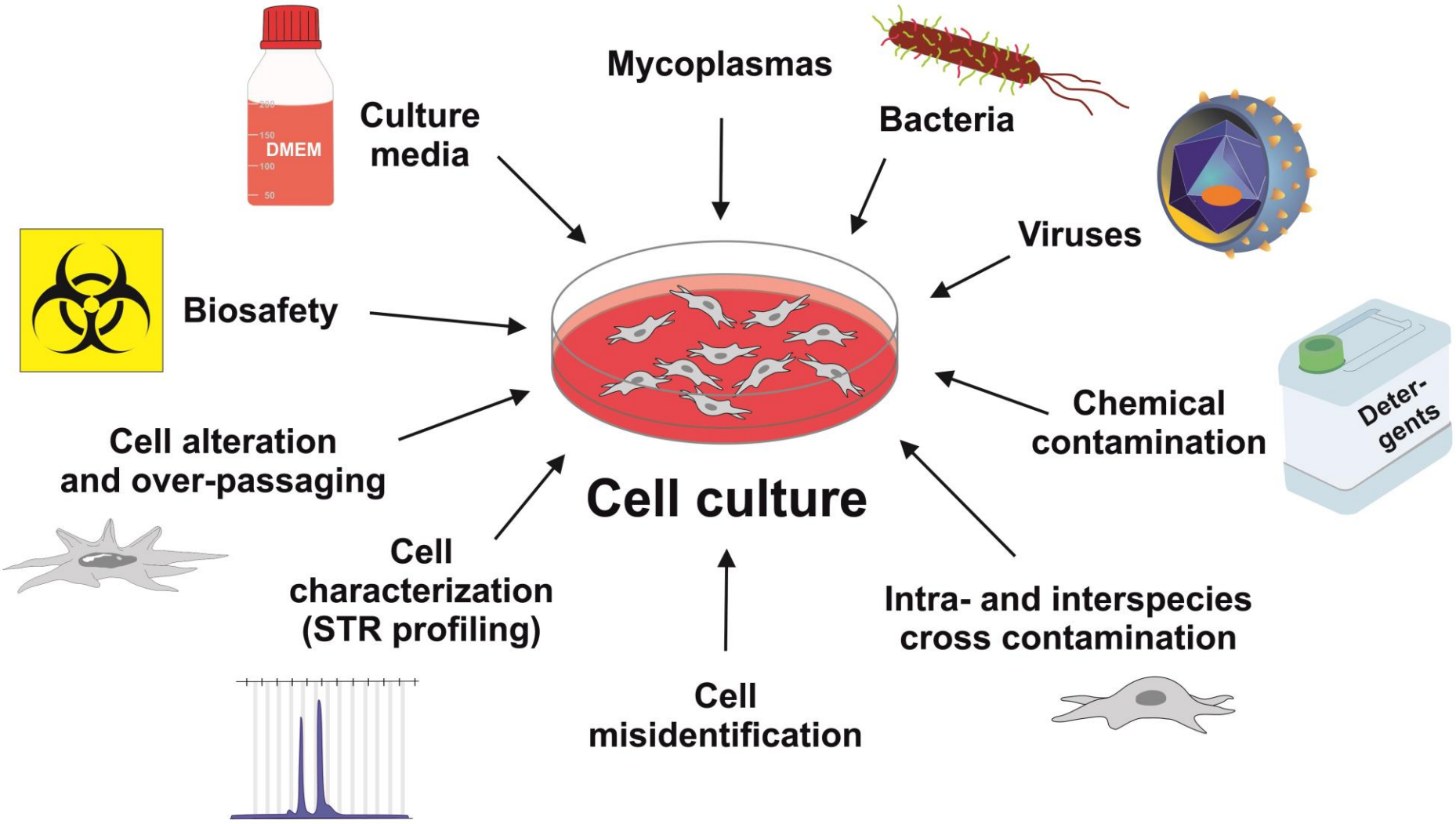
> 730 strains
Plant viruses
> 830 viruses
Human & Animal Cell Lines
> 880 cell lines
Plasmids
> 250 plasmids
Fungi & Yeasts
> 8,000 strains
Bacteriophages
> 1,000 phages
Protists
coming soon

Prices

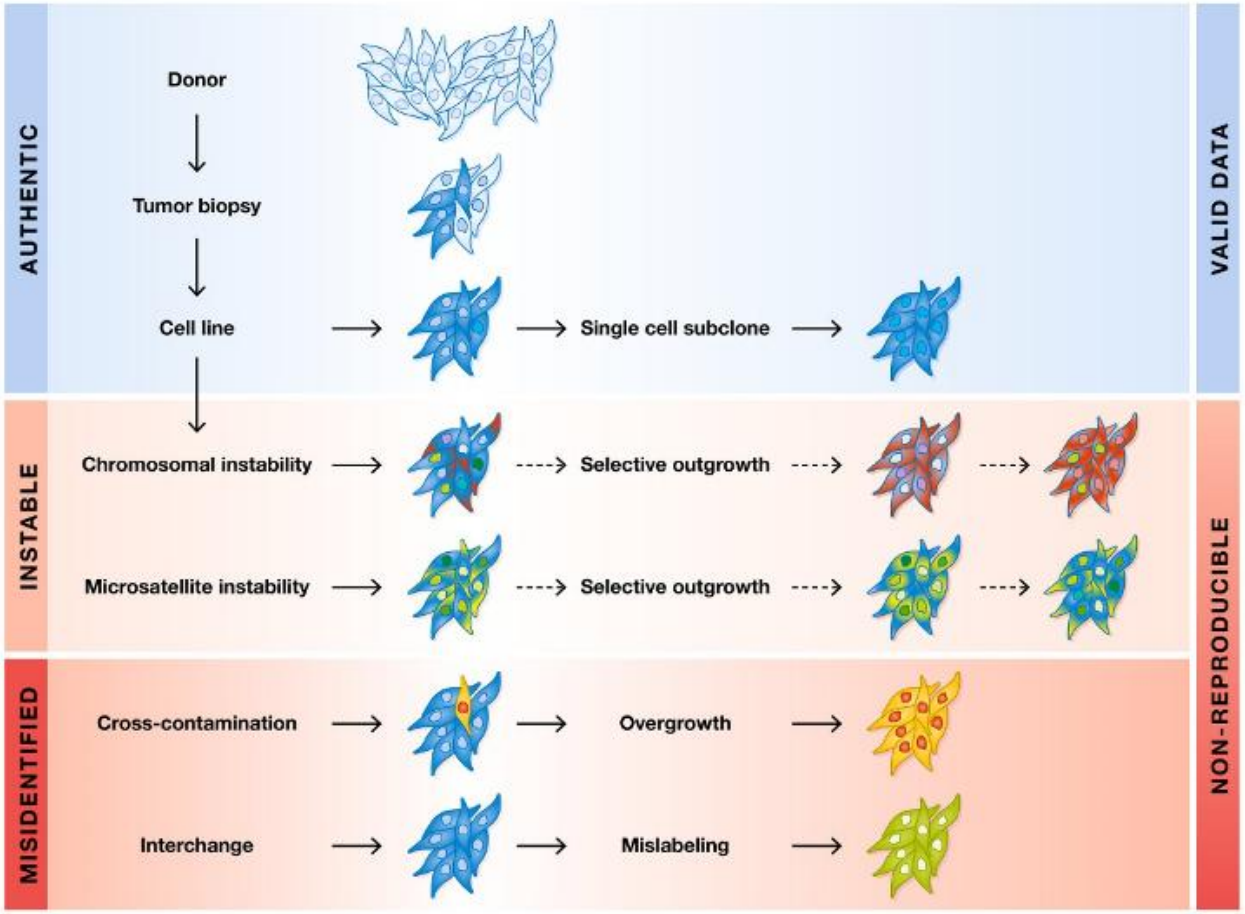
Price List Human and Animal Cell Lines

Cell lines	Frozen culture (one ampoule)	400 €
	Growing culture	800 €
DNA	DNA isolated from cell lines of the cell culture collection per 25 µg	500 €
	DNA isolated from cell lines of the cell culture collection per 5 µg	120€


DMEM
Culture media
Mycoplasmas
Bacteria
Viruses
Biosafety
Chemical contamination
Deter-gents
Cell alteration and over-passaging
Cell culture
Cell characterization (STR profiling)
Cell misidentification
Intra- and interspecies cross contamination

Why Does Cell Line Authentication Matter?

Misidentification and contamination of biological models—including cell lines, organoids, and homograft and xenograft models—remains a challenge.

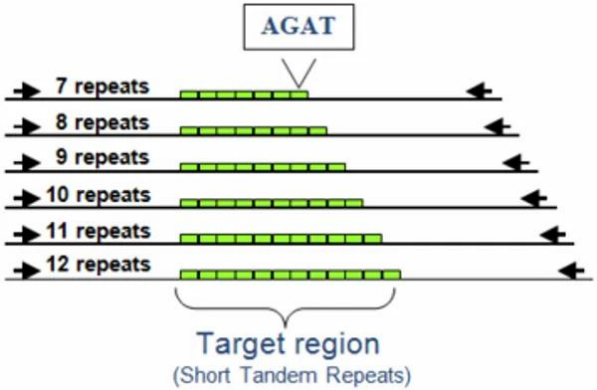


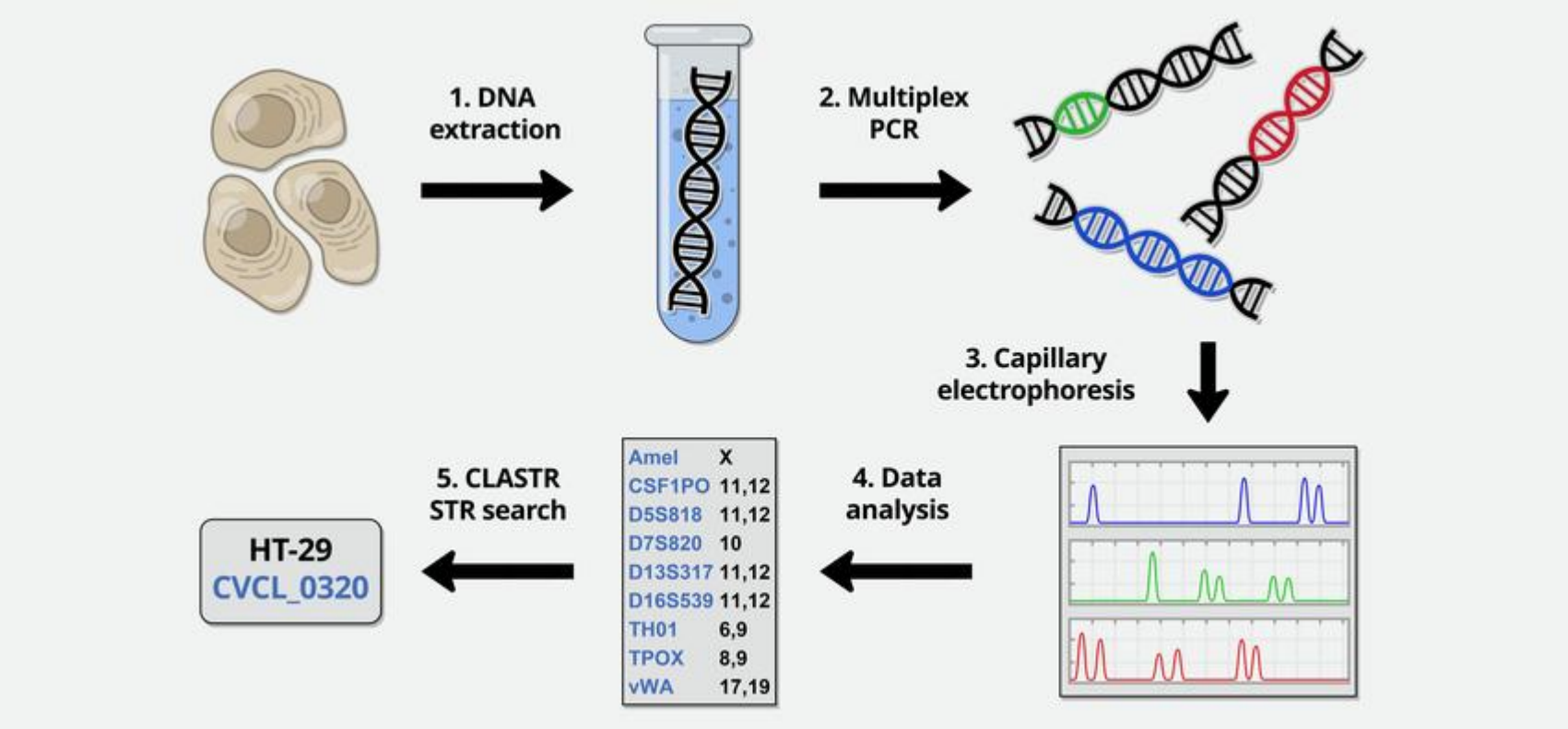
Studies indicate that **up to 33% of popular cell lines** are contaminated by intra-/interspecies cells, mycoplasma, and/or viruses, and the International Cell Line Authentication Committee (ICLAC) currently lists **530+ misidentified cell lines** that have no known authentic stock.

Everything was fine until they discovered their HeLa cell line expressed Y chromosome markers.

Cell Line Authentication Using STR Analysis

The analysis of Short Tandem Repeats (STR) has become the standard for intra-species identity testing of human cell lines.





Biosafety in laboratory settings

The Centers for Disease Control (CDC) in the United States sets regulations and recommendations for biosafety in laboratory settings. Cell culture laboratories can be assigned to one of four biosafety levels based on the risk associated with handling a particular agent.

Biosafety Level 1 (BSL-1)

is the **basic level of protection** and is appropriate for agents that **do not cause disease in normal, healthy humans.**

BSL-1

Low risk to personnel and the environment

Biosafety Level 2 (BSL-2)

is appropriate for moderate-risk agents that can cause human disease through ingestion, inhalation, or injection.

Most cell culture labs should be at least BSL-2.

BSL-2

Moderate risk to personnel and the environment

Biosafety Level 3 (BSL-3)

is appropriate for agents that have the **potential for aerosol transmission** and may cause serious and potentially lethal infections.

BSL-3

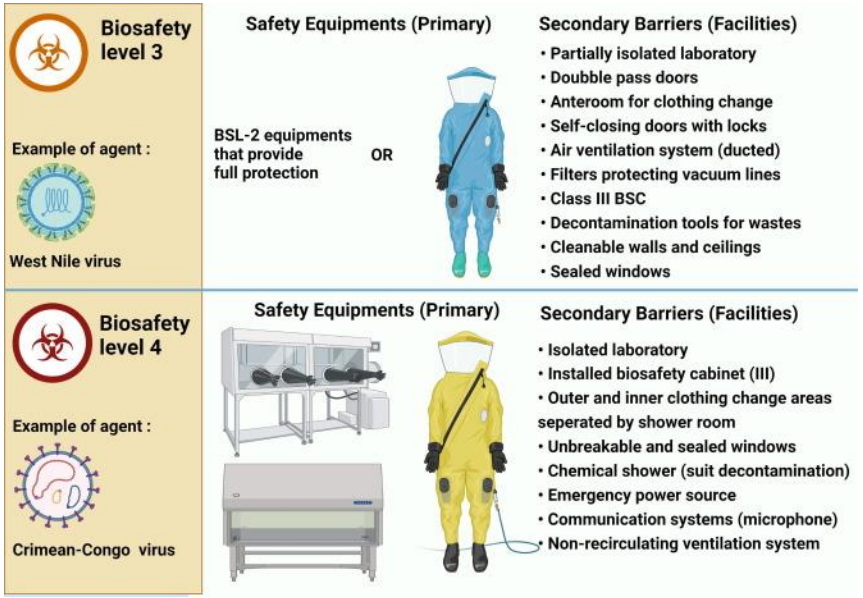
Serious disease for human, animal or plant (not spread by casual contact)

Biosafety Level 4 (BSL-4)

is the highest level of containment and is appropriate for agents that pose **a high individual risk of life-threatening disease** through infectious aerosols and for which no treatment is available. These agents are restricted to high-containment laboratories.

BSL-4

Very serious disease for human, animal or plant (often untreatable)



CO_2 Incubator

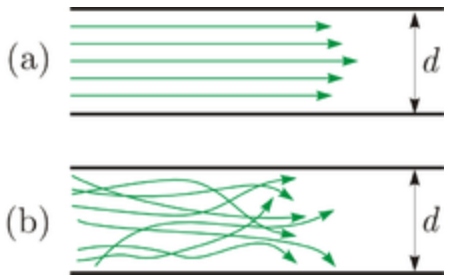
La cappa per colture cellulari

I filtri HEPA (*High Efficiency Particulate Air filter)*
e la tecnologia del **flusso laminar**e hanno reso possibile la realizzazione di "**clean rooms**", di laboratori microbiologici di massima sicurezza, di cappe sterili e di cabine "Biohazard'.

- Il flusso laminare è ottenuto dalla combinazione di un **filtro assoluto** (HEPA) con una massa d'aria che lo attraversa alla velocità costante di di 0,45 m/sec. (+/- 20%).

- **Il flusso laminare** è un flusso d'aria unidirezionale formato da filetti d'aria sterili paralleli che si muovono alla medesima velocità in tutti i punti, così da creare una corrente d'aria omogenea senza turbolenze.

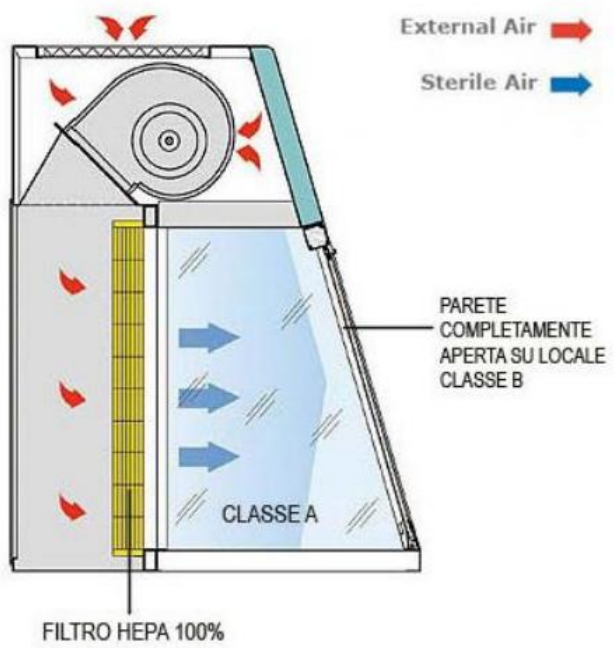
- In un ambiente sterile così ottenuto ogni contaminante libero nella zona di **lavoro viene trascinato lontano da un fronte di aria sterile**.



Filtrazione a flusso laminare

Se la priorità è **proteggere dalla contaminazione il prodotto**,
si opterà per una cabina a **flusso laminare orizzontale**,

Cappe a Flusso Laminare: ORIZZONTALE



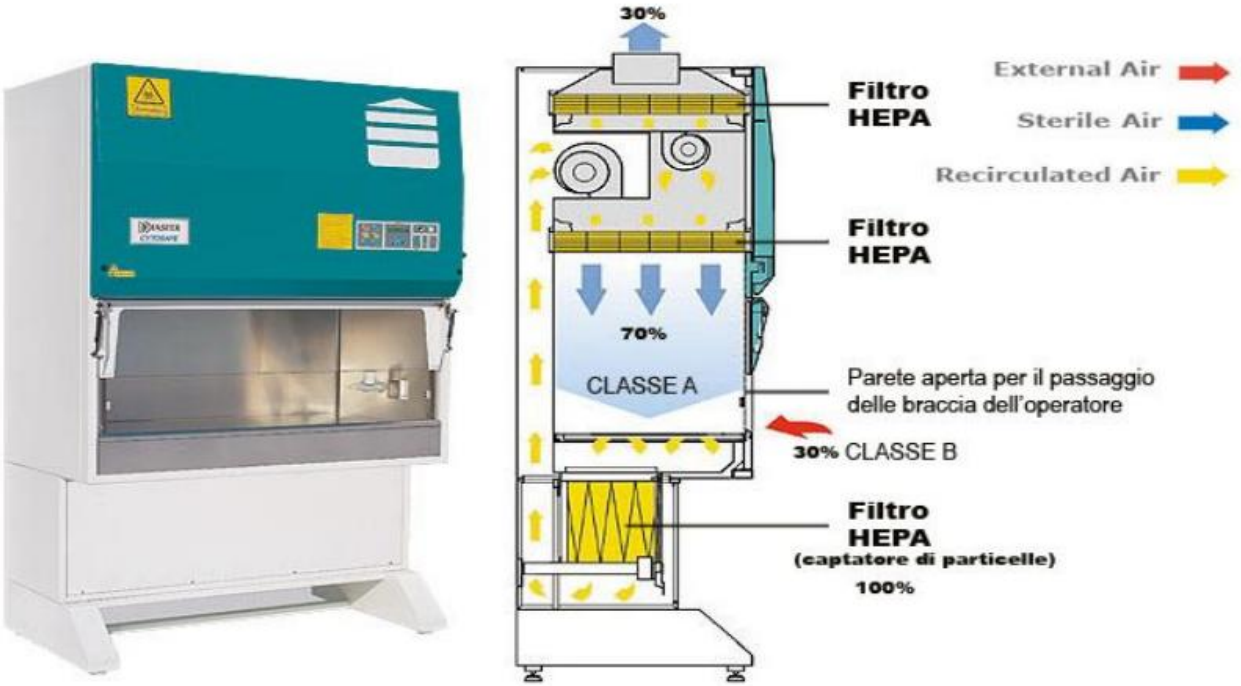
Cappa a flusso laminare ORIZZONTALE per manipolazione prodotti sterili:
NON protegge il personale

8

Filtrazione a flusso laminare

Se bisogna **proteggere anche l'operatore** la scelta deve cadere su di una **cappa a flusso laminare verticale.**

Cappe a Flusso Laminare: VERTICALE

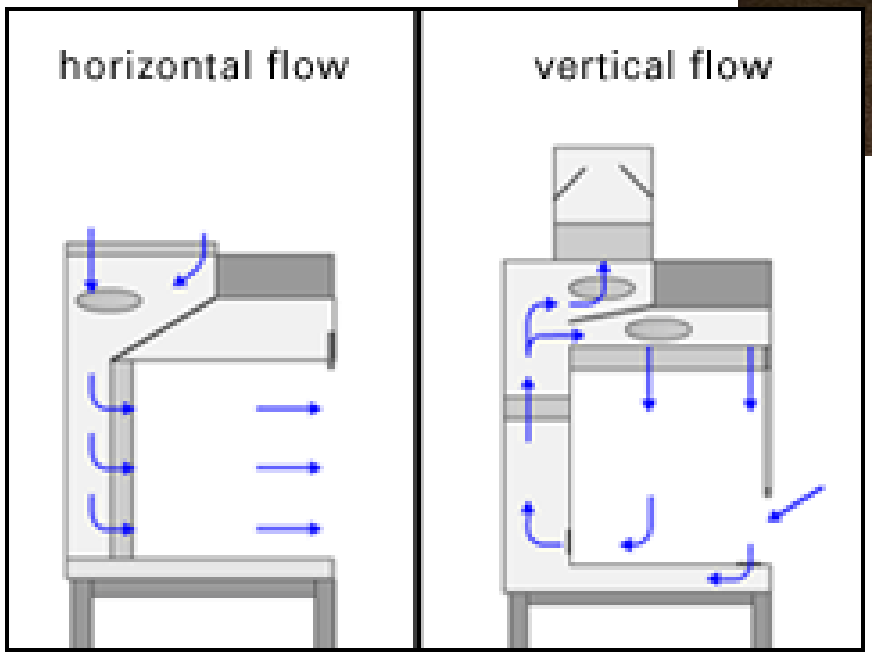


Cappa a flusso laminare VERTICALE, per manipolazione prodotti sterili citotossici, a protezione del prodotto e del personale

Se invece si manipola materiale patogeno, bisogna garantire la protezione di prodotto, operatore e ambiente con apposite cappe di sicurezza contro il rischio biologico (**Biohazard**)


JANUS


VBH72


horizontal flow
vertical flow

Sterilità

Al fine di mantenere la sterilità per la coltura cellulare, il laboratorio di biologia cellulare deve essere esclusivo: occorre lavorare sotto cappe a flusso laminare, in cui vanno sostituiti periodicamente i filtri.

- Per la sterilizzazione dei materiali:
  - **Stufa a secco**, 150°C per 3 ore → per la vetreria
  - **Autoclave** (calore umido), 1 atm, 121°C → per filtri, soluzioni saline…
  - **Filtrazione con filtri da 0.22 µm** → terreni di coltura, soluzioni organiche…
  - **Raggi γ** → materiali di plastica

Precauzioni per la prevenzione delle contaminazioni

Per prevenire le contaminazioni occorre seguire alcune regole:

1) i terreni e le soluzioni che si usano devono essere tutti **sterili**;

2) si aggiunge **penicillina-streptomicina** per evitare contaminazioni da batteri; anfotericina B (se non tossica per le cellule) contro i miceti;

3) si destina un laboratorio solo alle colture cellulari;

4) si opera sempre sotto cappa a flusso laminare;

5) si utilizza solo materiale sterile (di vetro o di plastica);

6) si utilizzano pipettatori elettrici;

7) la cappa va pulita **a inizio e fine lavoro**;

8) i filtri della cappa vanno periodicamente controllati;

9) l'incubatore va pulito periodicamente;

Contaminazioni delle colture

- L’ingresso indesiderato nel terreno di coltura di microrganismi è dannoso: questi competono con le cellule in coltura (che generalmente hanno ritmo di crescita inferiore) e possono secernere sostanze tossiche. Tipici contaminanti sono **batteri, micoplasmi, lieviti, muffe.**

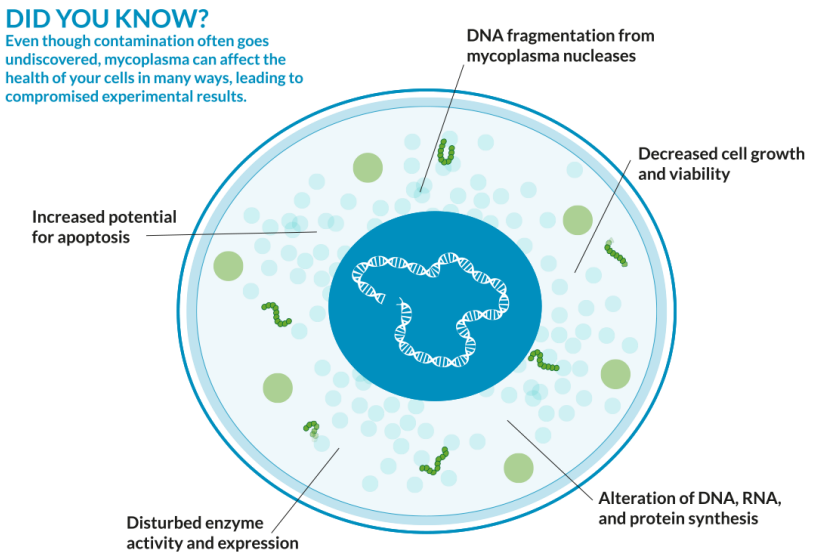
- In caso di infezione batterica il **terreno vira in fretta (acidifica) e intorbidisce**. Le **infezioni da micoplasmi** sono più subdole, in quanto il microrganismo è meno visibile e richiede un paio di settimane per formare colonie visibili.

- Questo pericolo determina la prassi di aggiungere antibiotici al terreno (ad esempio: streptomicina-penicillina); va ricordato che sono stabili per pochi giorni a 37°C.

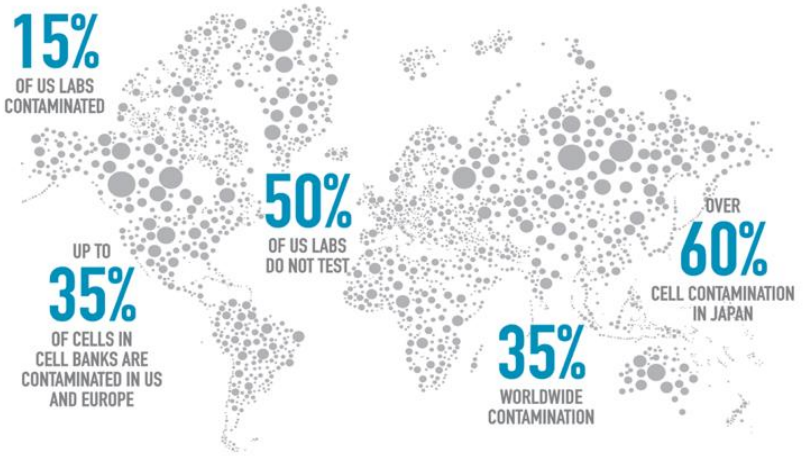
Mycoplasma contamination in cell cultures

Mycoplasma are one of the most common and elusive contaminants of mammalian cell cultures.

As the **smallest known free-living organism**, mycoplasma are a pervasive, parasitic species of highly infectious bacteria that **are resistant to most antibiotics, including the most commonly used penicillin and streptomycin**.



They are estimated **to contaminate between 15–35% of all continuous cell cultures worldwide**, and while over 180 species have been identified since mycoplasma was first isolated in 1956, only **six species are responsible for 95% of all infections in cell cultures.**



https://www.bioind.com/worldwide/mycoplasma-contamination-in-cell-cultures/

The extracellular medium

Adhesion substrate

Temperature

Mammal cells grow at 37°C, saturated with humidity

Gas phase

Mammal cells typically grow at 5% CO2

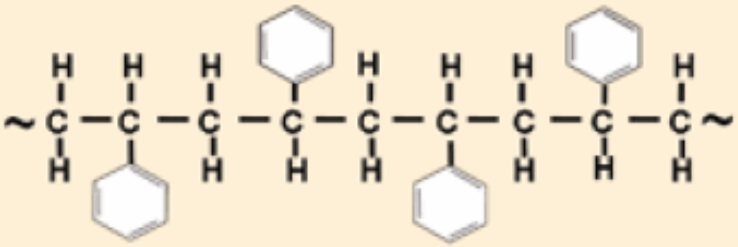
Culture **Medium**

Cell culture plastics

Most of the vessels are manufactured from **polystyrene**, a long carbon chain polymer with benzene rings attached to every other carbon.

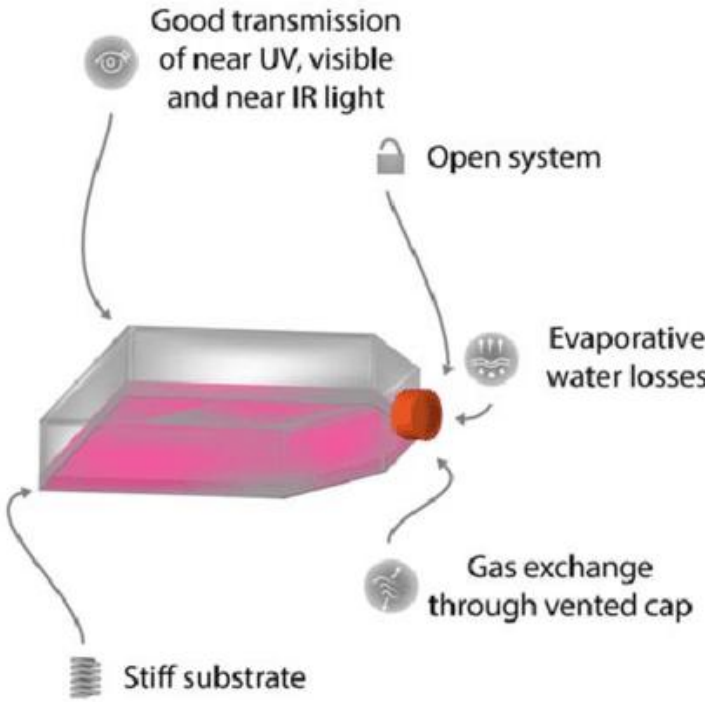
Polystyrene was chosen because:
- it has excellent optical clarity,
- is easy to mold
- can be sterilized by irradiation.

.



However, it also has **one significant drawback**—it is a very **hydrophobic** (nonwettable) polymer to which cells have difficulty attaching

Tissue Culture Polystyrene



Catalog #	Name	Size	Price (EUR)	Qty
156499	Nunc™ EasYFlask™ Cell Culture Flasks, T75, filter	Case of 100	153,00	
156472	Nunc™ EasYFlask™ Cell Culture Flasks, T75, Solid	Case of 100	148,00	

Add to cart

CLS3335 SIGMA

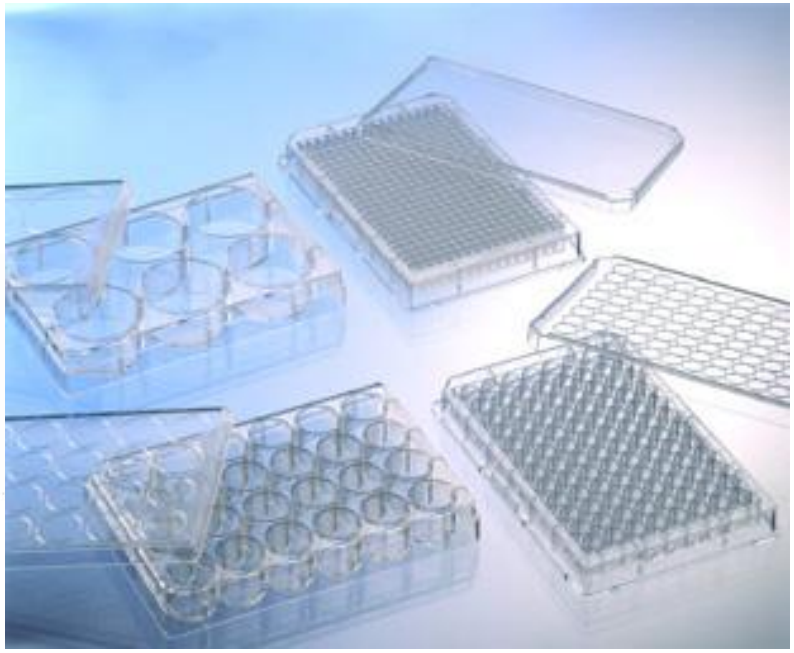
Corning® CellBIND® Multiple Well Plate

size 6 wells, clear bottom flat, sterile, lid

Synonym: **6 well culture plate, cell culture multiwell plates, cell culture plates**

SDS | SIMILAR PRODUCTS

eCl@ss 32190102



Acquisto | Sicurezza e Documentazione

Proprieta'

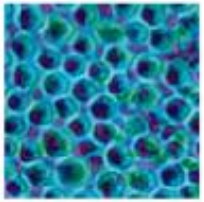
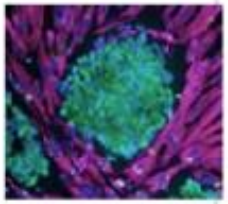
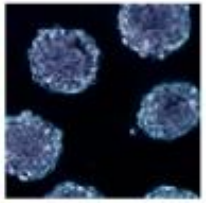
Related Categories	Cell Culture Supplies, Cell Culture Surfaces, CellBind Surface, Corning, Corning 6-Well Microplates, Altro...
material	**clear bottom flat**
	clear flat bottom wells

Prezzo e disponibilità

SKU-Confezionamento	Disponibilità	Prezzi (EUR)	Quantita'
CLS3335-5EA	**Stima per la spedizione in 09.04.18**	22.90	0
CLS3335-50EA	**Solo 3 a stock (altri in arrivo)** - DA	163.70	0

Find your cell culture plastics by surface

To help ensure optimal results for different types of cell culture and the subsequent cellular analysis we offer a range of cell culture surfaces, spanning a variety of formats. Let us help guide your selection by starting with the cell culture surfaces modified to suit the needs of your specific cell types.

Nunclon Delta for adherent cells	Non-treated for suspension cultures or general assays	Nunclon Vita for fastidious cells	Nunclon Sphera for spheroid-organoid culture	Nunclon UpCell for adherent culture plus trypsin-free cell harvesting	Poly-D-Lysine and Collagen I for primary and finicky cells
					
A standard tissue culture (TC) surface modification that makes the polystyrene surface more hydrophilic, thus facilitating maximum adhesion for a broad range of cell types.	High quality, optically clear virgin polystyrene with hydrophobic surface, ideal for suspension cell culture, also useful for a variety of biochemical binding assays.	Unique energy treated surface that when combined with conditioned media supplemented with a ROCK inhibitor, can support attachment and expansion of human pluripotent stem cells.	Enables formation of reproducible spheroid cultures. Cells grow and aggregate with virtually no cell attachment to the culture vessel. For spheroid culture, organoid culture, and 3D culture.	Enables harvesting of cells in single cell suspension or as contiguous cell sheet by temperature reduction and can create 3D tissue models without foreign-to-the-body scaffold material	ECM coated surface mimicking the growth environment inside the body, ideal for cells that have difficulties growing on the regular tissue culture surface. Collagen I is of animal origin, whereas PDL is fully synthetic.

The extracellular medium

Adhesion substrate

Temperature

Mammal cells grow at **37°C**, saturated with humidity

Gas phase

Mammal cells typically grow at **5% CO_2**

Culture Medium

The extracellular medium

Adhesion substrate

Temperature

Mammal cells grow at **37°C**, saturated with humidity

Gas phase

Mammal cells typically grow at **5% CO_2**

Culture Medium

The Six Main Ingredients in Cell Culture Medium

There are six main ingredients found in cell culture media:

1.Buffering system

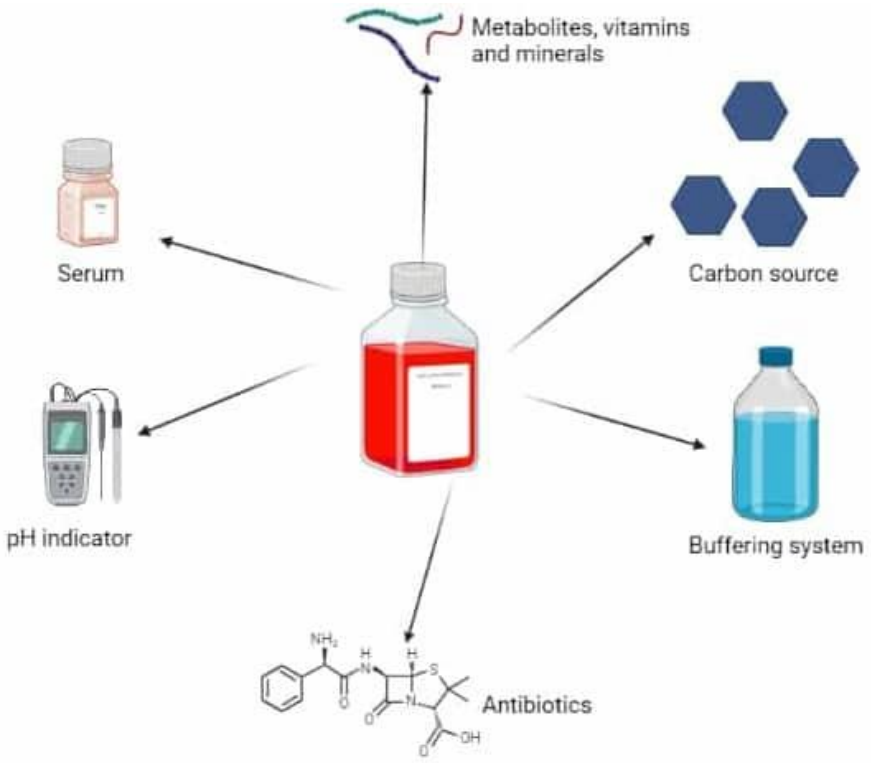
2.pH Indicator (e.g., phenol red).

3.Carbon source (e.g., glucose).

4.Metabolites, vitamins, and minerals.

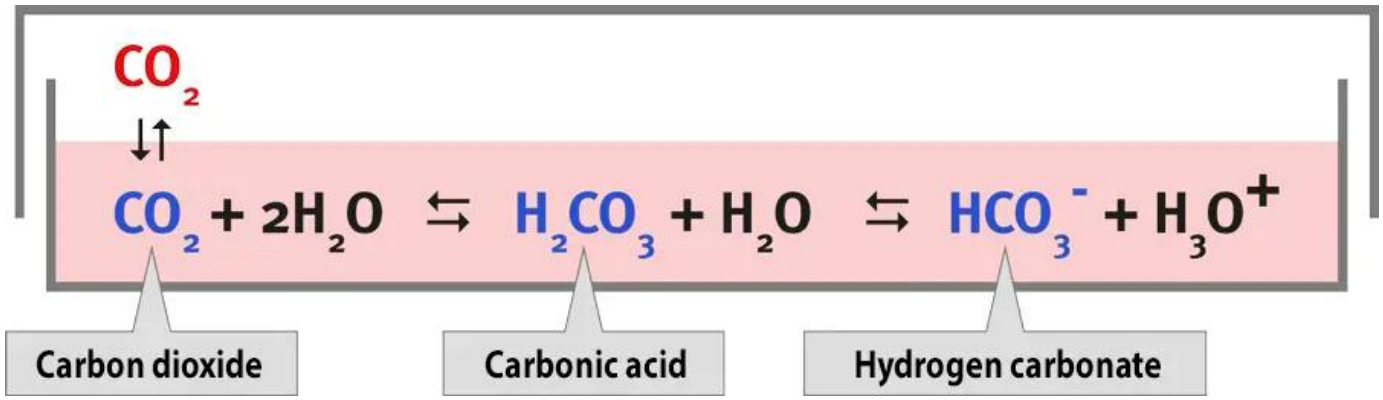
5.Antibiotics.

6.Serum.



For growth, cells require a medium **pH between 7.2 and 7.4**.

To maintain this pH, incubators with a gas phase containing **5% CO_2** and media containing **$NaHCO_3$** are most commonly used.



In aqueous solution, **bicarbonate** dissociates and undergoes hydrolysis (reforming the original weak acid).

$$NaHCO_3 + H_2O$$
$$\updownarrow$$
$$Na^+ + HCO_3^- + H_2O$$
$$\updownarrow$$
$$Na^+ + H_2CO_3 + HO^-$$
$$\updownarrow$$
$$Na^+ + H_2O + HO^- + CO_2$$

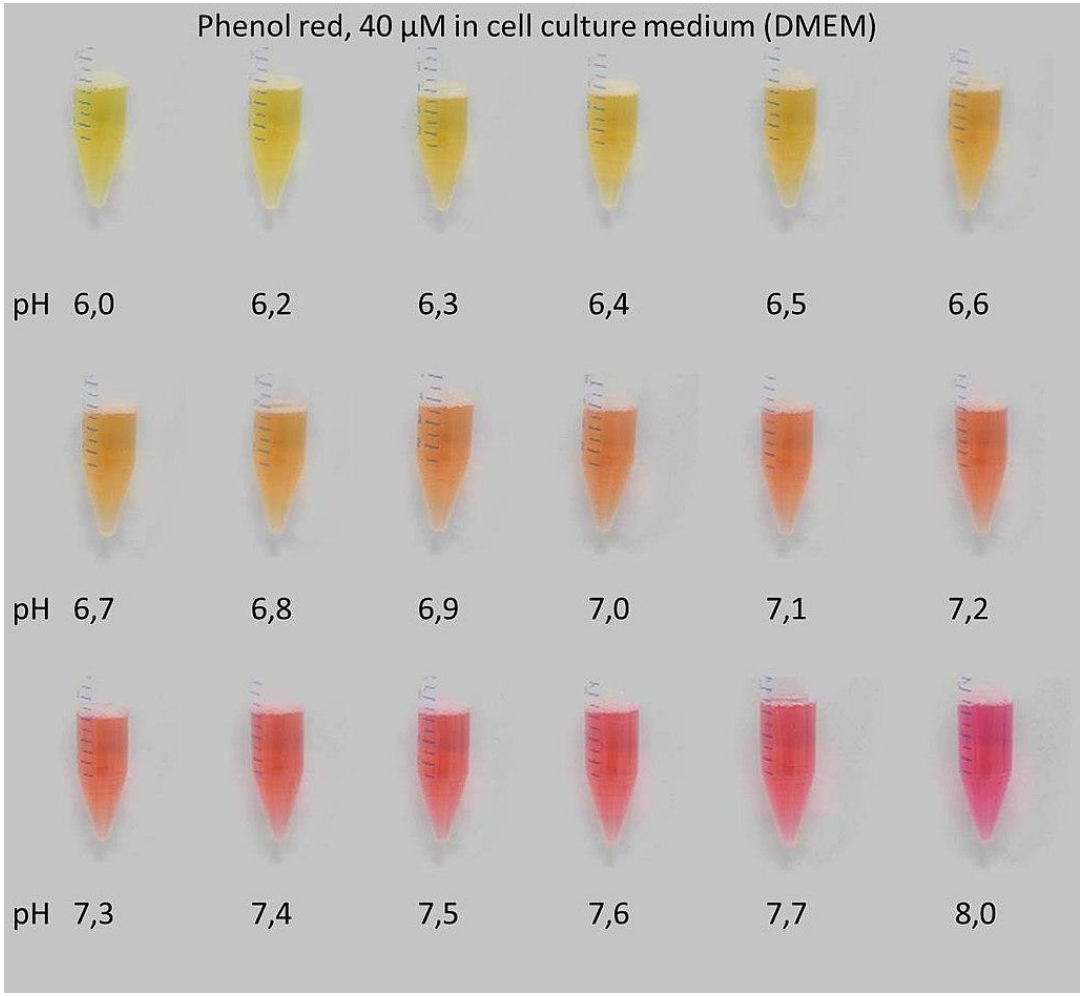
The CO2 present in the incubator tends to counteract this increase,thus maintaining the correct pH of the medium.

Per avere un indicazione visiva del pH, i terreni vengono addizionati **di rosso fenolo**, un indicatore che ha un colore

giallo-arancio a **pH acido**

rosso-arancio a pH **7,3,**

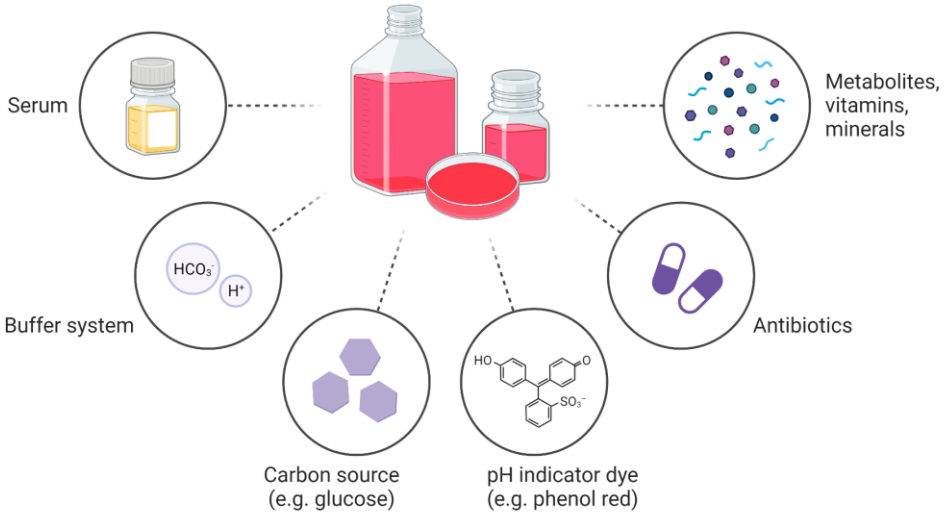
al **rosso viola** a **pH alcalino**.



Con **il proliferare della crescita cellulare**, in incubatore al 5% di CO_2, il terreno tenderà al giallo a causa dell'acidificazione prodotta dal metabolismo cellulare.

Se le piastre tenute in incubatore, invece, virano al violaceo, significa che la regolazione della CO_2 del macchinario è errata oppure che le cellule stanno morendo (non sono metabolicamente attive).

Components of Cell Culture Media



The Six Main Ingredients in Cell Culture Medium

There are six main ingredients found in cell culture media:

1.Buffering system

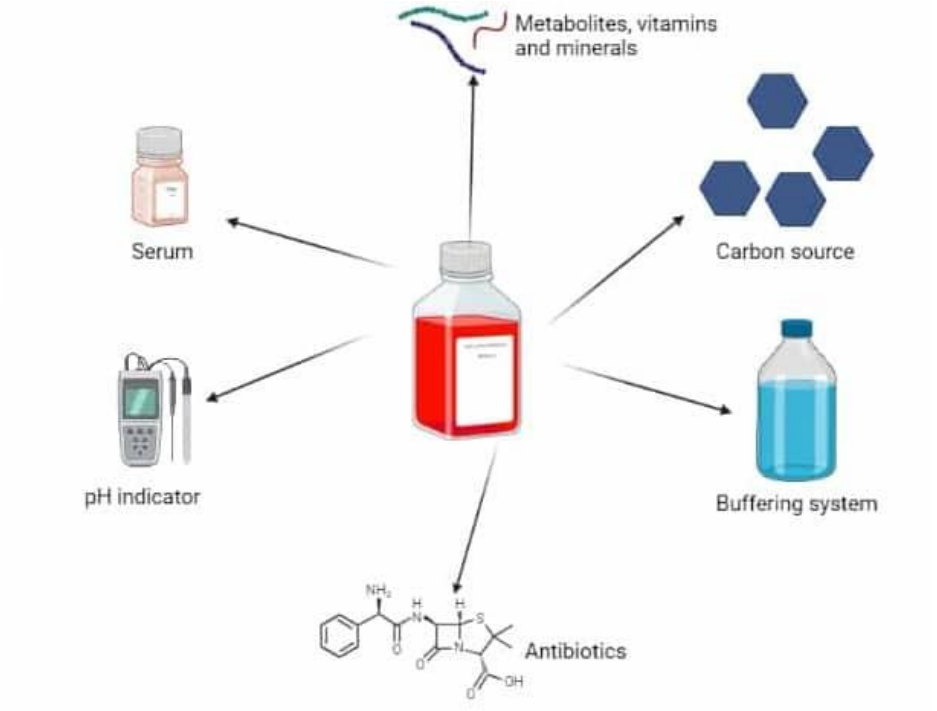
2.pH Indicator (e.g., phenol red).

3.Carbon source (e.g., glucose).

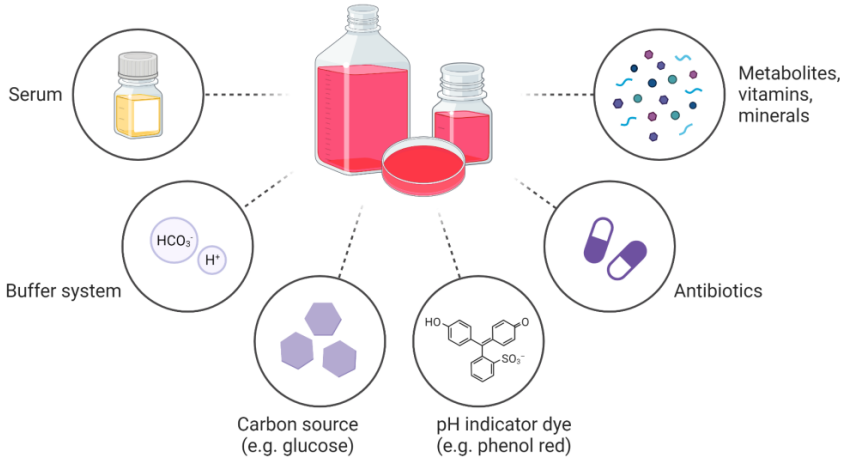
4.Metabolites, vitamins, and minerals.

5.Antibiotics.

6.Serum.



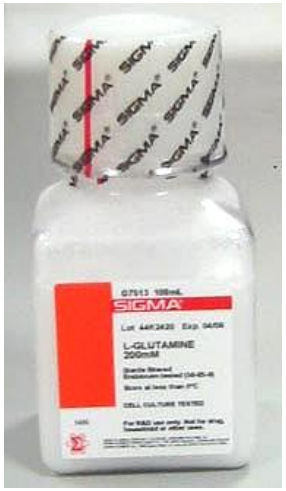
Components of Cell Culture Media



The composition of the culture media includes:

- Inorganic salts (Na+, K+, Mg++, Ca++, Cl-, etc.)
- Glucose, Glutamine Essential amino acids
- B vitamins
- Trace amounts of Fe, Zn, Cu, Se, Mn, and Mo (trace amounts mean they do not need to be added, as minimal contamination from other components is sufficient).
- Fetal bovine serum, 5-20% or a substitute

Cell culture environment (*in vitro*)



Supplements

L-glutamine

- Essential amino acid (not synthesised by the cell)
- Energy source (citric acid cycle), used in protein synthesis
- Unstable in liquid media - added as a supplement

GlutaMAX supplement is a dipeptide, L-alanine-L-glutamine, which is more stable in aqueous solutions and does not spontaneously degrade.

Non-essential amino acids (NEAA)

- Usually added to basic media compositions
- Energy source, used in protein synthesis
- May reduce metabolic burden on cells

Growth Factors and Hormones (e.g.: insulin)

- Stimulate glucose transport and utilisation
- Uptake of amino acids
- Maintenance of differentiation

Antibiotics and Antimycotics

- Penicillin, streptomycin, gentamicin, amphotericin B
- Reduce the risk of bacterial and fungal contamination
- Cells can become antibiotic resistant – changing phenotype
- Preferably avoided in long term culture

Terreno Eagle modificato di Dulbecco - alto glucosio

Sinonimo/i: DME, DMEM

Tutte le immagini (1)

Confronta	N° Catalogo	Descrizione	SDS	Determinazione del prezzo
☐	D5796	With 4500 mg/L glucose, L-glutamine, and sodium bicarbonate, without sodium pyruvate, liquid, sterile-filtered, suitable for cell culture	⬇	Nascondi ^

SKU	Taglio della confezione	Disponibilità	Prezzo	Quantità
D5796-500ML	500 mL	1 Disponibile per la spedizione il 14 marzo 2024 Dettagli...	~~32,70 €~~ 24,52 €	− +
D5796-1L	1 L	1 Disponibile per la spedizione il 14 marzo 2024 Dettagli...	~~50,00 €~~ 37,50 €	− +
D5796-6X500ML	6 x 500 mL	1 Disponibile per la spedizione il 14 marzo 2024 Dettagli...	~~179,00 €~~ 134,25 €	− +

Aggiungi al carrello

DMEM - Dulbecco's Modified Eagle Medium

‹ Cell Culture Media

DMEM - Dulbecco's Modified Eagle Medium

DMEM/F12, Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12

F10 Nutrient Mixture

Ham's F12 Nutrient Mixture

Media 199

MEM, Minimum Essential Media

RPMI Medium 1640

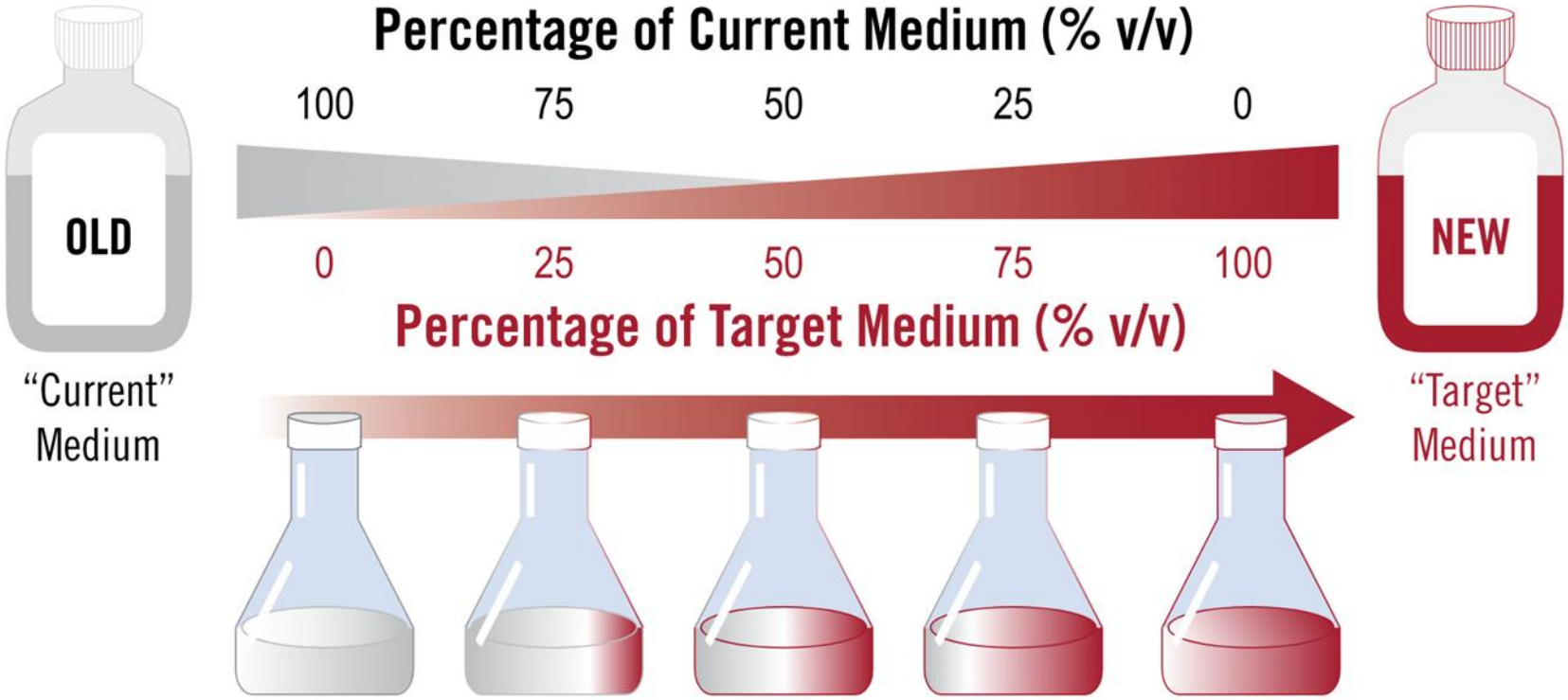
Gibco® DMEM delivers superior quality, consistency and control for your mammalian cell culture.

- View all results or choose the right DMEM below

Choose the Right DMEM

Glucose	Glutamine	Phenol Red	Format
• High Glucose • Low Glucose • No Glucose	• GlutaMAX™ • L-Glutamine • No Glutamine	• Phenol Red • No Phenol Red	• Liquid • Powder

Media Adaptation



CARATTERISTICHE FISICHE DEI TERRENI e INFLUENZE DELL'AMBIENTE

- pH
- Tampone
- Osmolarità
- Temperatura → 37°C in incubatore
- Natura del substrato

FASE GASSOSA:
95% O_2 5% CO_2

✓ Contenitori di plastica
✓ Contenitori o supporti di vetro

Piastra Petri per colture cellulari

Medium

Cellule adese al substrato


F6178 500 ML
SIGMA
Fetal Bovine Serum
Sterile filtered
Store at -20 C
For Research Use Only.
Not for drug, household or other uses.
MSDS Available
F6178 500 ML
SIGMA
Fetal Bovine Serum
Sterile filtered
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MSDS Available
F6178 500 ML
SIGMA
Fetal Bovine Serum
Sterile filtered
Store at -20 C
For Research Use Only.
Not for drug, household or other uses.
MSDS Available

Cell culture environment (*in vitro*)

Foetal Calf/Bovine Serum (FCS & FBS)

- Growth factors and hormones
- Aids cell attachment
- Binds and neutralise toxins
- Long history of use

- Infectious agents (prions)
- Variable composition
- Expensive
- Regulatory issues (to minimise risk)

Heat Inactivation (56°C for 30 mins) – why?

- Destruction of complement and immunoglobulins
- Destruction of some viruses (also gamma irradiated serum)

Care! Overdoing it can damage growth factors, hormones & vitamins and affect cell growth

The use of serum is controversial. Fetal serum is generally preferred because it contains fewer antibodies.

Benefits

- Protects membranes thanks to the correct level of viscosity;
- Introduces growth factors and hormones;
- Carries lipids, iron, and organic molecules;
- Promotes cell-substrate interactions;
- Contains trypsin inhibitors

Disadvantages

- Possible antibody toxicity;
- Lot-to-lot variability;
- Metabolic inhibitors;
- Growth factors that favor fibroblasts over other cell types
- ~~€€€~~ $$$$
- FBS South American origin 500ml 107.00 EURFBS Australian origin 500ml 439.00 EUR

- Gibco FBS - ISIA Traceability Certified and Fingerprinting Origin Guarantee

Gibco Cell Culture Media

Cell Culture Reagents

Antibiotics

Serum-Free Media

Specialty Media

Media Supplements

Recombinant Proteins

Cancer Cell Culture

value, and innovation that have made Gibco products a global market leader for over 50 years.

Limited-time online offer—get 23% off Specialty FBS, order now

Gibco fetal bovine serum products

Choose the right fetal bovine serum for your specific cell culture needs—from basic research to specialty assays. Whether you need fetal bovine serum with the least viral risk, the lowest endotoxin levels, or sera qualified for specialty applications and assays, Gibco products offer superior value.

Value FBS

Sera for standard research applications

- Up to 50 quality tests including 9CFR virus testing, endotoxin, performance
- Triple 0.1 micron filtration*

Select products

Premium FBS

Sera with the least risk of BSE and lower viral risk

- Meets USP/EP guidelines
- Up to 90 quality tests including EMA virus testing; USP/EP mycoplasma, endotoxin, performance; biochemical/hormonal profiling; Oritain fingerprinting
- Triple 0.1 micron filtration

Select products

Specialty FBS

Sera qualified for specialty research

- Specific assays, including stem cell research, immunoassays, antibodies, and others

Select products

Gibco™

Fetal Bovine Serum, qualified, United States

Catalog number: 26140079

Related applications: Mammalian Cell Culture

Contact us for support ›

	Catalog number	Unit Size	Price (EUR)	Availability	Qty
☆	26140079 also known as 26140-079	500 mL	538,00 Your price: Sign In	***	
☆	A3160502	10 x 50 mL	636,00 Your price: Sign In	***	
☆	26140087 also known as 26140-087	100 mL	145,00 Your price: Sign In	***	
☆	A3160501	50 mL	67,25 Your price: Sign In	***	

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science/cell-culture/cell-culture-products.html?TablePage=9628642

ion L... Overview of protein ... Pfizer vuole il botox ...

Product #	Product Name	Origin	Treatment	Hemoglobin	Endotoxin	Availability
12003C	Fetal Bovine Serum Australia origin, USDA approved, sterile-filtered, suitable for cell culture	Australia origin		Hemoglobin, ≤25 mg/dL	≤10 EU/mL endotoxin	
F7942	Fetal Bovine Serum Canada origin, sterile-filtered, γ-irradiated, suitable for cell culture NEW	Canada origin		Hemoglobin, ≤20 mg/dL	≤10 EU/mL Endotoxin	
F1051	Fetal Bovine Serum Canada origin, sterile-filtered, suitable for cell culture	Canada origin		Hemoglobin, ≤20 mg/dL	≤10 EU/mL endotoxin	
12105C	Fetal Bovine Serum Dialyzed by ultrafiltration against 0.15M NaCl, USA origin, sterile-filtered, cell culture tested	USA origin	Dialyzed by ultrafiltration against 0.15M NaCl	Hemoglobin, ≤20 mg/dL	≤10 EU/mL endotoxin	
F9665	Fetal Bovine Serum Heat Inactivated, non-USA origin, sterile-filtered, suitable for cell culture	non-USA origin	Heat Inactivated	Hemoglobin, ≤25 mg/dL	≤10 EU/mL endotoxin	available only in EU
12203C	Fetal Bovine Serum New Zealand origin, sterile-filtered, suitable for cell culture	New Zealand origin				
F6765	Fetal Bovine Serum USA origin, Charcoal Stripped, sterile-filtered, suitable for cell culture	USA origin	Charcoal Stripped	Hemoglobin, ≤20 mg/dL	≤10 EU/mL endotoxin ((typically ≤1 EU/ml))	
F0392	Fetal Bovine Serum USA origin, Dialyzed by ultrafiltration against 0.15	USA	Dialyzed by ultrafiltration	Hemoglobin,	≤10 EU/mL	

Serum-free, chemically defined media

are specialized cell culture solutions lacking animal-derived serum (like FBS) and containing only known, purified components

The use of **serum-free media** stems from the desire for controlled, reproducible, and cell-specific conditions.

They must contain: a trypsin inhibitor, adhesion factors, hormones, growth factors, nutrients, and proteins.

Advantages

Greater reproducibility;

Standardization even between different laboratories;

Can favor specific subpopulations (not just fibroblasts);

Facilitates product purification.

Disadvantages

Cells are more sensitive to environmental conditions;

Slower growth;

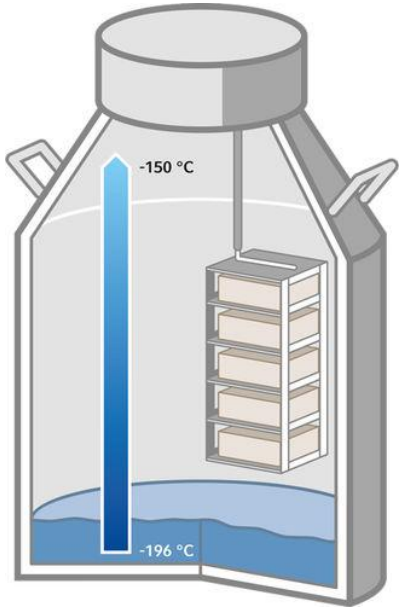
Some additives are expensive and/or unstable;

Media are specific to a specific cell type.

Cell preservation: cryopreservation

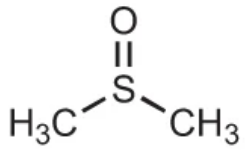
Freezing in liquid nitrogen (at **-196°C**) is the preferred method for most eukaryotic cells.

under these conditions **cell viability is maintained** for the period during which the sample is stored in liquid nitrogen.



Cell preservation: cryopreservation

In order to moderate cryopreservation-induced damage, cryoprotective agents (CPAs) are employed, the most common being dimethyl sulfoxide (DMSO) and glycerol.



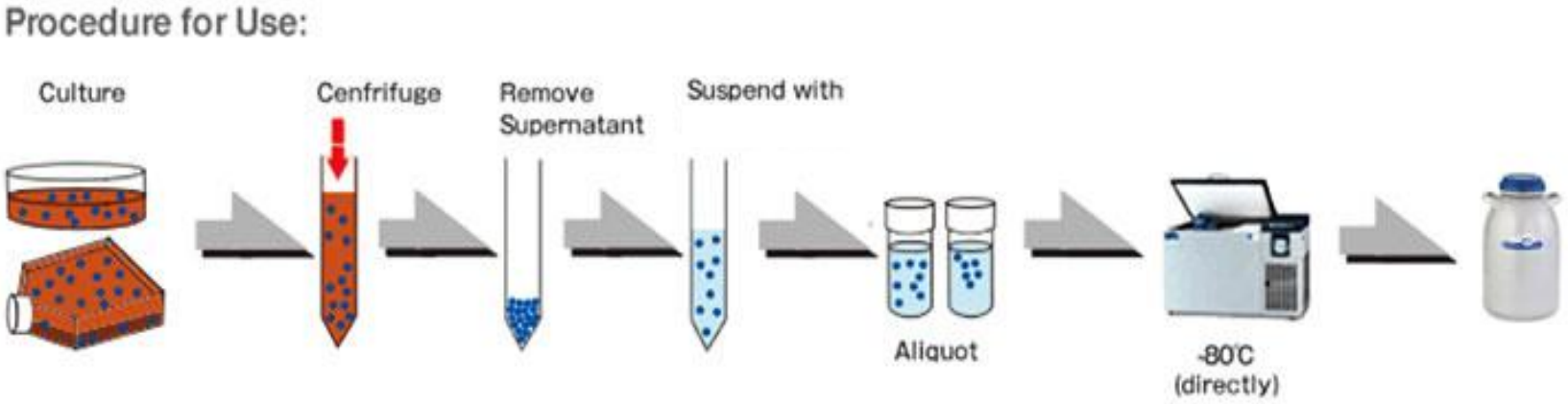
DMSO

Dimethyl sulfoxide (DMSO) is widely used for the freezing of cells in cell culture instead of glycerol.

DMSO is added to **prevent the formation of ice crystals** during the freezing process, otherwise cells would be destroyed.

Usually, such freeze medium consists of standard **culture medium supplemented with 10% DMSO**.

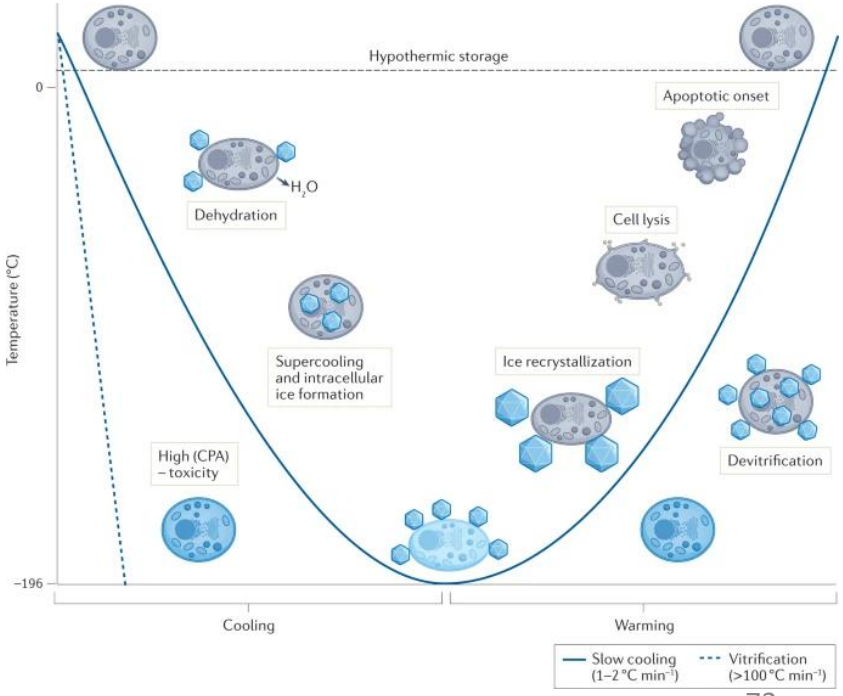
Some researchers use **90% serum supplemented with 10% DMSO** as freeze medium, which enhances the viability in some cell types



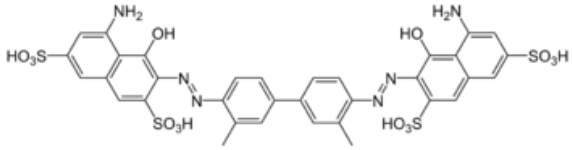
The **speed** at which the sample is frozen is very important - **slow decrease** in temperature

Gradual cooling involves first keeping the sample on ice, then **slowly reaching temperatures of -70°C** and then the sample is transferred to liquid nitrogen.

Thawing, on the contrary, **must be rapid**: the sample is transferred to a thermostated bath at 37°C for a few minutes and the cells are resuspended in complete medium for plating.

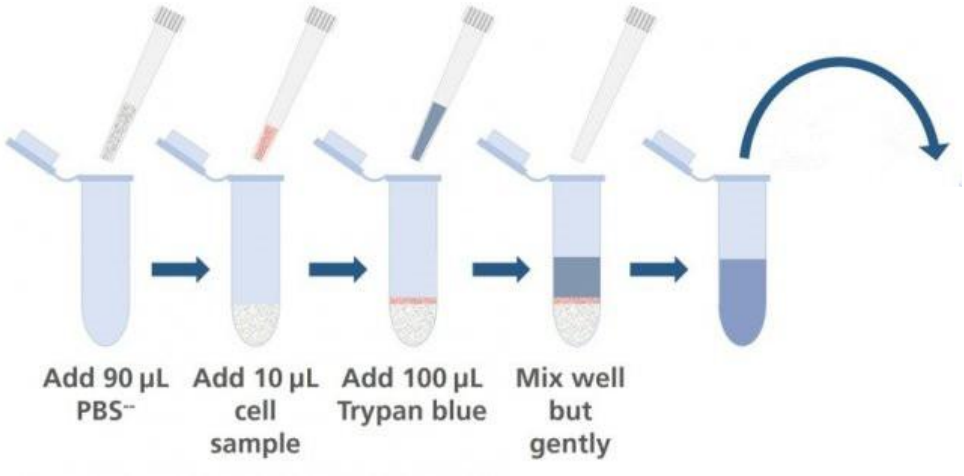


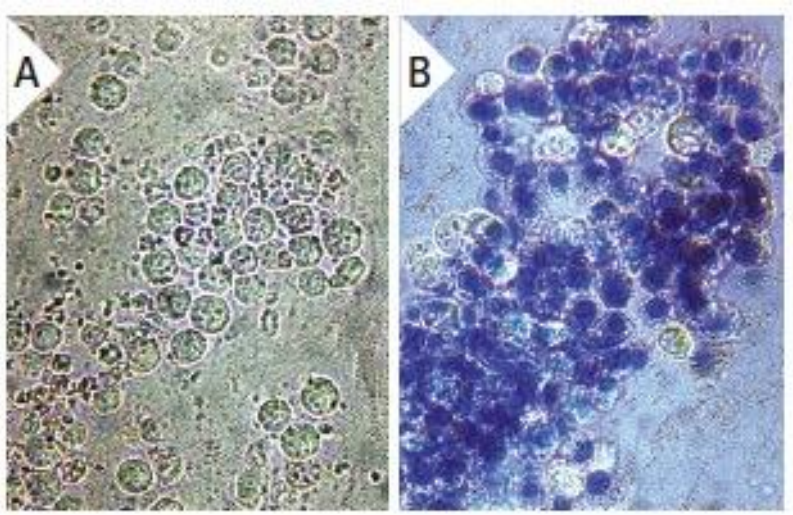
Cell viability test



To discriminate between live and dead cells, **Trypan blue** is used

dye that is taken up only by **dead cells**





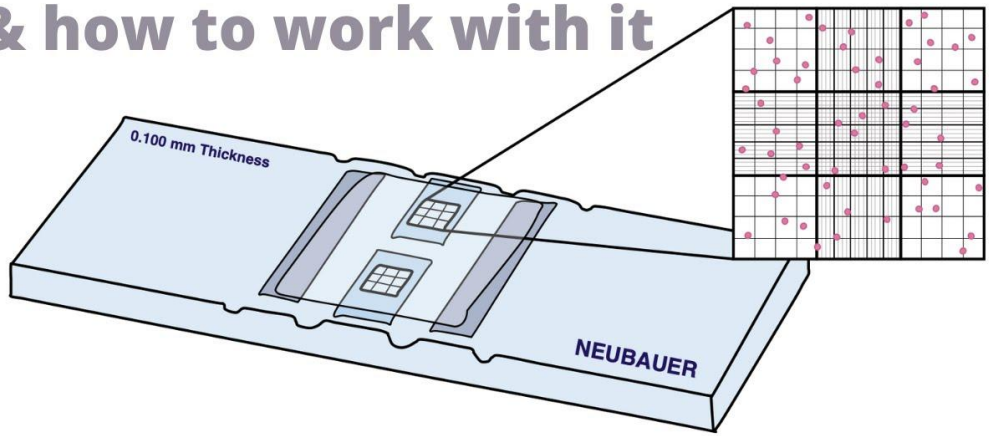
- The cells are resuspended in PBS
- add the Trypan Blue solution
- leave a few minutes at room temperature.

- counted in Burker's chamber

In this way, the **percentage of dead cells** present in the examined sample is obtained.

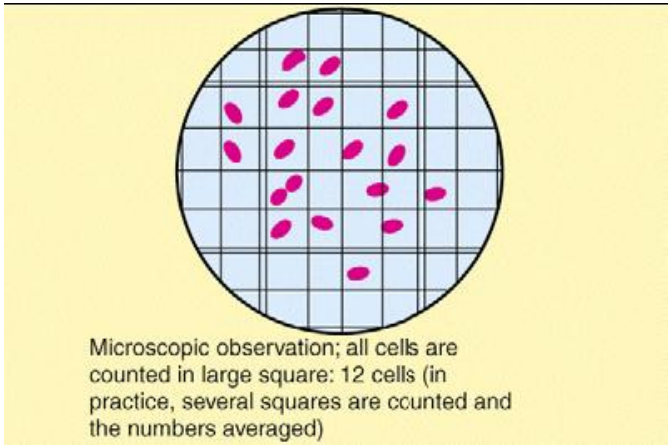
Counting Chamber

& how to work with it



Per effettuare il conteggio:
dopo aver pulito la camera, montata correttamente, depositato un dato volume di cellule, al microscopio si contano le cellule presenti nei quadrati delimitati da una doppia barra e quelle presenti su due lati dello stesso quadrato (non si contano, invece, quelle su gli altri due lati).

FORMULA DEL CONTEGGIO IN CAMERA DI BURKER:
(N° cellule in n1) x 10^4 x FD = cellule/ml nella sospensione originaria n1 è tutto il quadrato grande della Burker, costituito da 16 quadrati piccoli, e due bordi interni a scelta, escludendo le cellule che cadono sulle righe della zigrinatura


Microscopic observation; all cells are counted in large square: 12 cells (in practice, several squares are counted and the numbers averaged)

https://www.youtube.com/watch?v=pP0xERLUhyc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