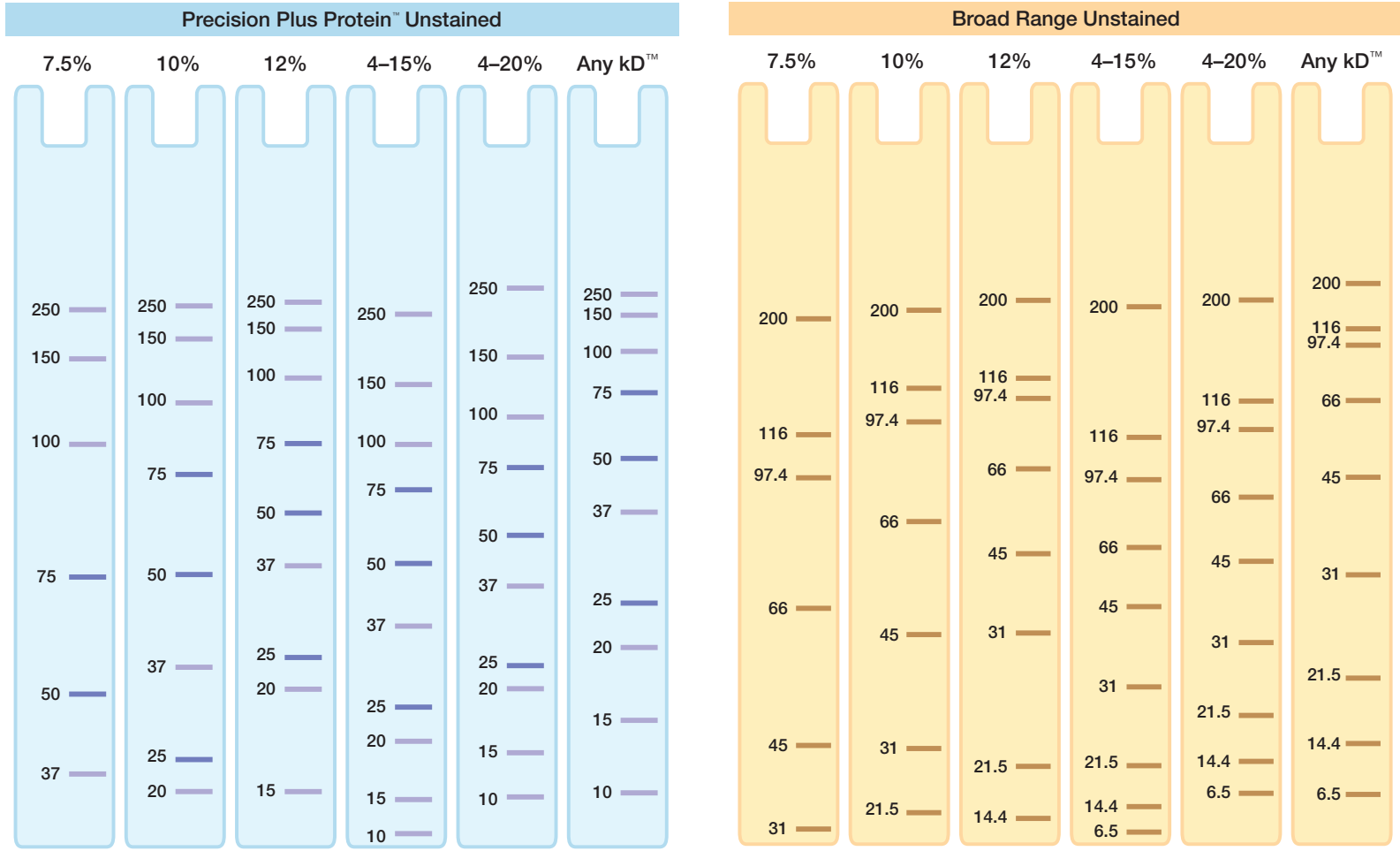
INDICAZIONI PER L'ESPERIENZA di LABORATORIO

Mini-PROTEAN precast gels are composed of polyacrylamide with a bisacrylamide crosslinker, and they are available in a range of formulations (Table 1.1) and in a selection of single percentages and gradients.

Table 1.1. Mini-PROTEAN precast gel formulations.

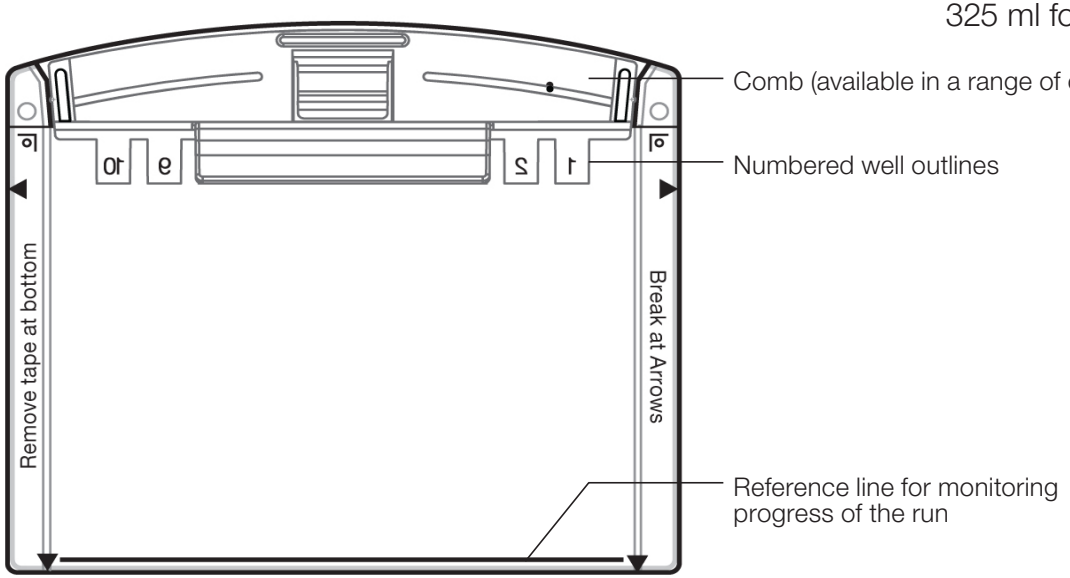
Application	Gel Formulation	Sample Buffer	Running Buffer
SDS-PAGE	Mini-PROTEAN TGX™ Mini-PROTEAN TGX Stain-Free	Laemmli	Tris/glycine/SDS
Native PAGE	Mini-PROTEAN TGX Mini-PROTEAN TGX Stain-Free	Native	Tris/glycine
Peptide analysis	Mini-PROTEAN Tris-Tricine	Tricine	Tris/Tricine/SDS
dsDNA separation	Mini-PROTEAN TBE	Nucleic acid	Tris/boric acid/EDTA (TBE)
ssDNA and RNA separation	Mini-PROTEAN TBE-urea	TBE-urea	TBE



Migration charts for protein standards on Mini-PROTEAN TGX and Mini-PROTEAN TGX Stain-Free gels.

QUALE SISTEMA ELETTROFORETICO UTILIZZEREMO???

Gel material	Polyacrylamide
Gel dimensions	7.2 x 8.6 cm
Gel thickness	1.0 mm
Resolving gel height	6.2 cm (5.6 cm for 50 µl well)
Cassette dimensions	8.5 x 10 cm
Cassette material	Styrene copolymer
Comb material	Polycarbonate
Running buffer	750 ml for 1–2 gels, 1,000 ml for 3–4 gels (Mini-PROTEAN Tetra cell) 325 ml for 1–2 gels (Mini-PROTEAN II or Mini-PROTEAN 3 cell)



See Appendix B for buffer formulations. Do not adjust pH.

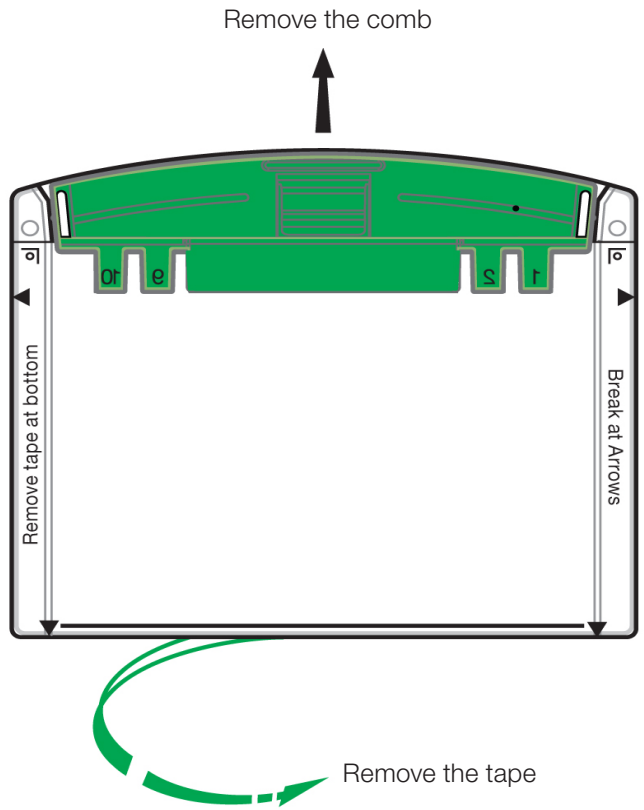
Running buffer (1x)	25 mM Tris, 192 mM glycine, 0.1% SDS Dilute 100 ml 10x stock (catalog #161-0732) with 900 ml deionized water (diH_2O).
Sample buffer (5X)	312,5 mM Tris-HCl, pH 6.8, 5 % SDS, 50% (v/v) glycerol, 0.025% bromophenol blue 25% β-mercaptoethanol or 250 mM DTT (added fresh) Use Laemmli sample buffer (catalog #161-0737) and add β-mercaptoethanol or DTT before use.
Sample buffer (4x)	250 mM Tris-HCl, pH 6.8, 4% LDS, 40% (w/v) glycerol, 0.02% bromophenol blue, 15% beta-mercaptoethanol or 200 mM DTT (added fresh) Use 4x Laemmli sample buffer (catalog #161-0747) and add β-mercaptoethanol or DTT before use.

1. Remove the gels from the storage pouch and prepare them for assembly:

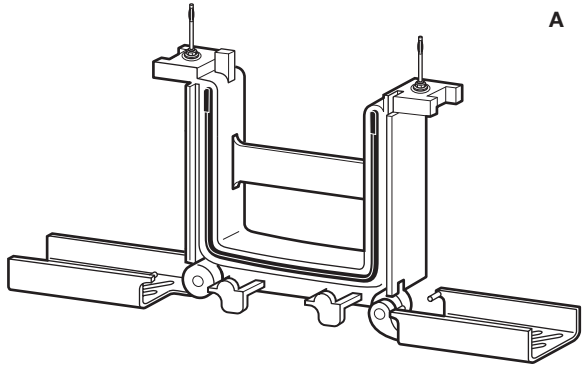
   a. Remove the comb: Position thumb on the indentation (middle of comb) and remove the comb by pulling upward in one smooth motion.

   b. Remove the tape: Pull gently to remove the green tape from the bottom of the cassette. If necessary, use the opening key or comb to help remove the tape at the corners.

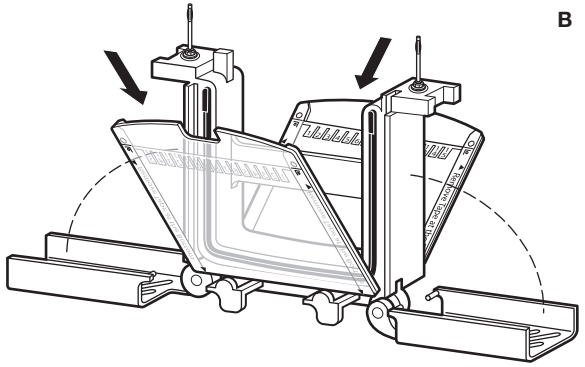
   c. Rinse the wells: Use a syringe, wash bottle, or disposable transfer pipet to rinse the wells with

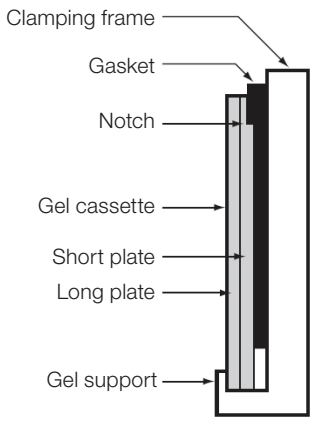


A

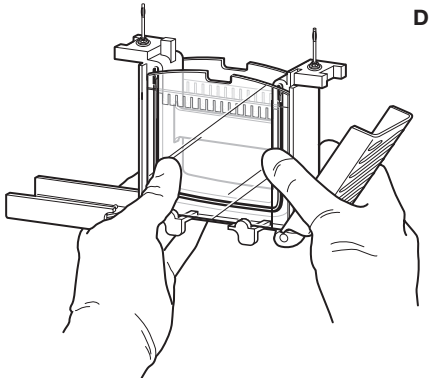


B

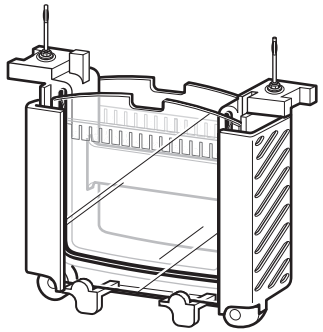



Clamping frame
Gasket
Notch
Gel cassette
Short plate
Long plate
Gel support

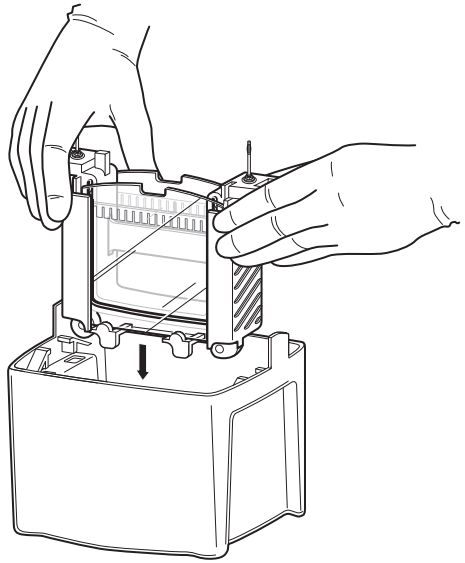
D



E



F



SDS-PAGE (Mini-PROTEAN TGX Gels)

Prepare Buffers

Running buffer (1x) Dilute 100 ml 10x stock (catalog #161-0732) with 900 ml diH_2O.

Sample buffer Use Laemmli sample buffers: catalog #161-0737 or catalog #161-0747 (4x).

▼

Prepare Gels and Assemble Electrophoresis Cell

Remove the comb and tape from the gels, rinse wells, and assemble the electrophoresis cell.

Fill the inner and outer buffer chambers with running buffer.

▼

Prepare and Load Samples

Component	Reducing	Nonreducing
Sample	5 µl	5 µl
Laemmli sample buffer, 2x (catalog #161-0737)	4.75 µl	5 µl
β-Mercaptoethanol	0.25 µl	—
Total volume	10 µl	10 µl

Heat samples at 90–100°C for 5 min (or at 70°C for 10 min).

Load the appropriate amount of sample on the gel.

▼

Perform Electrophoresis

Connect the electrophoresis cell to the power supply and perform electrophoresis according to the conditions in the table.

Table A.1. Running conditions for SDS-PAGE in the Mini-PROTEAN Tetra cell. Standard conditions are constant 300 V.

	100 V	200 V	300 V
Run time	85–95 min	30–40 min	15–20 min
Expected current (per gel)			
Initial	15–20 mA	25–50 mA	55–75 mA
Final	5–10 mA	20–31 mA	45–70 mA
Expected temperature	25°C	25–35°C	30–45°C
Outer buffer volume			
1–2 Gels	2-gel mark	2-gel mark	4-gel mark
3–4 Gels	4-gel mark	4-gel mark	4-gel mark[1]

[1] Requires the PowerPac™ HV or PowerPac Universal power supply.

1. After electrophoresis is complete, turn off the power supply and disconnect the electrical leads.
2. Remove the lid from the tank and remove the gels from the cell. Pour off and discard the running buffer.
3. To open the cassette, align the arrow on the opening lever with the arrows marked on the cassette and insert the lever between the cassette plates at indicated locations. Apply downward pressure to break each seal. Do not twist the lever.
4. Pull the two plates apart from the top of the cassette, and gently remove the gel.

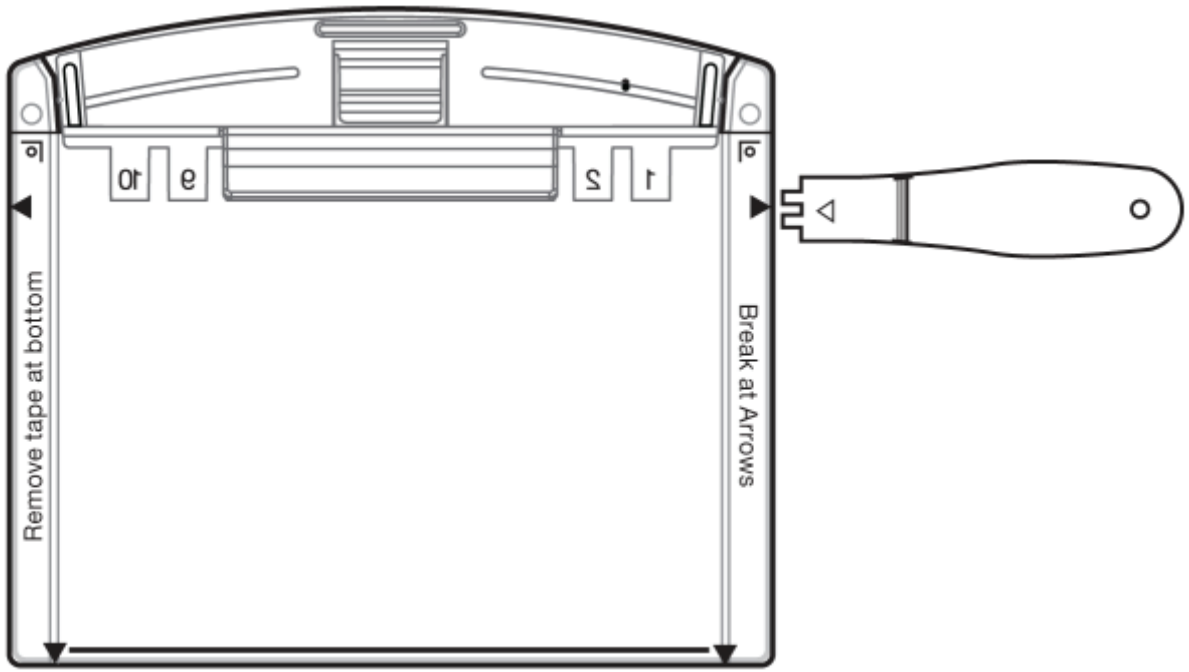
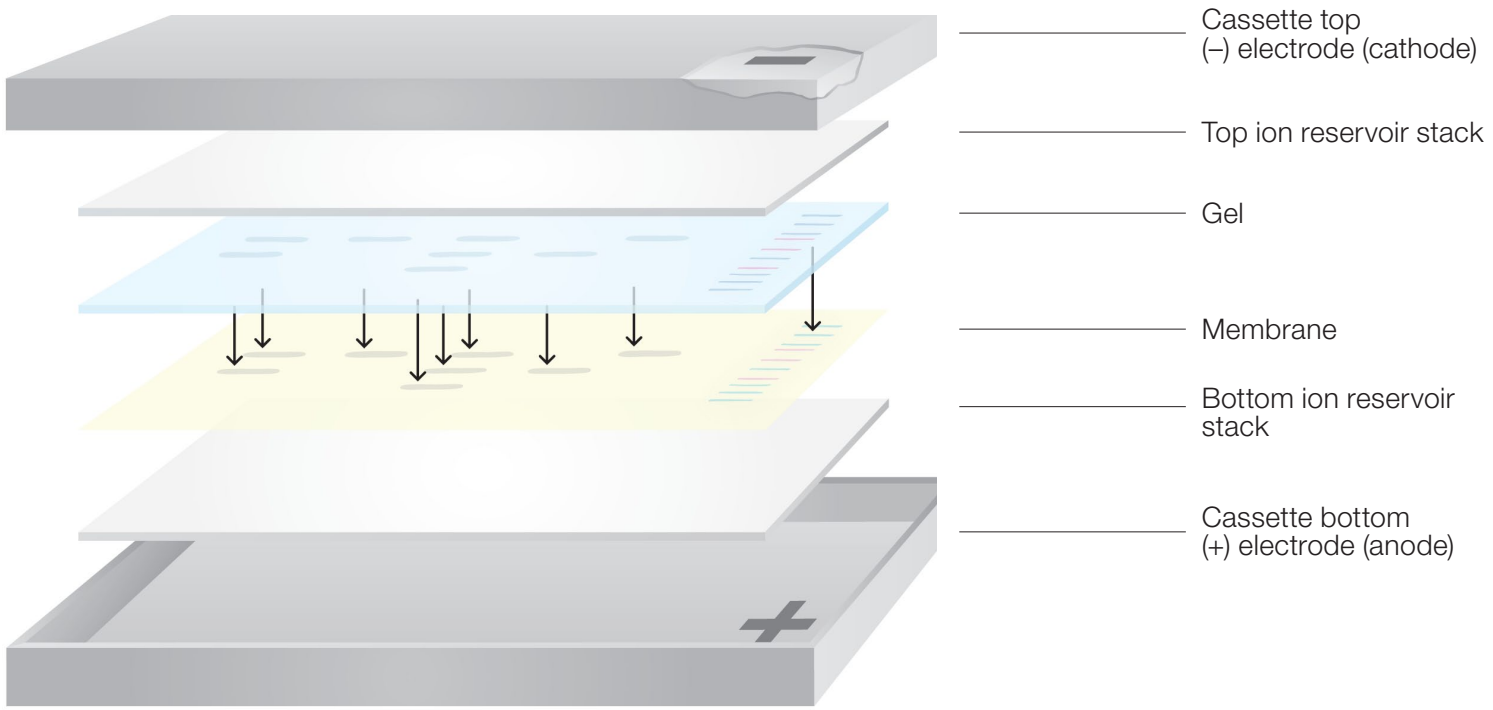


Table 3.1. Standard running conditions for SDS-PAGE in the Mini-PROTEAN Tetra cell.

Gel	Optimum Range	Run Conditions	Run Time
7.5%	40–200 kD		
10%	30–150 kD	300 V constant:	
12%	20–120 kD	Starting current (per gel): 55–75 mA	15–20 min
4–15%	20–250 kD	Final current (per gel): 45–70 mA	(Fill outer buffer volume
4–20%	10–200 kD		to the 4-gel mark)
Any kD	10–100 kD		

Table 3.3. PowerPac power supply recommendations.

# Gels	100 V	200 V	300 V
1–2	Basic/HC/HV/Universal	Basic/HC/HV/Universal	Basic/HV/Universal
3–4	Basic/HC/HV/Universal	Basic/HC/HV/Universal	HV/Universal



Assembly of the gel blot sandwich with the Trans-Blot Turbo system.

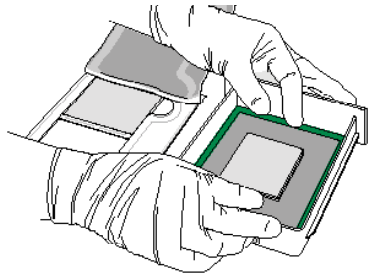
Table 11.2. Trans-Blot Turbo transfer protocols.

Protocol Name	MW, kD	Time, Min	1 Mini Gel	2 Mini Gels or 1 Midi Gel
STANDARD SD	Any	30	Up to 1.0 A, 25 V constant	Up to 1.0 A, 25 V constant
1.5 MM GEL	Any	10	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
HIGH MW	>150	10	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
LOW MW	<30	5	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
MIXED MW	5–150	7	2.5 A constant, up to 25 V	1.3 A constant, up to 25 V
1 Mini TGX	5–150	3	2.5 A constant, up to 25 V	N/A

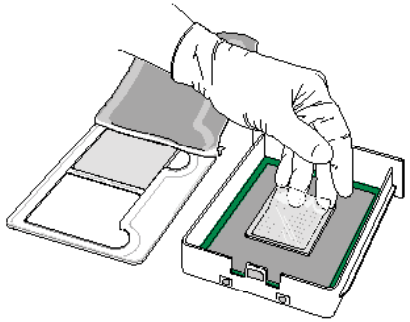
1

Mini Transfer Pack
(for one mini format gel)

Lay the ion reservoir stack with the membrane (anode stack) in the center of the cassette base. Ensure that the stack is not overlapping the green rubber molding in the base.

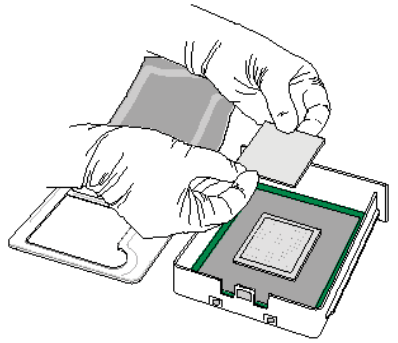


Carefully align the gel on the membrane. If necessary, gently use the blot roller to remove air bubbles between the gel and membrane. If transferring two mini gels, place them on the membrane so that the feet of the gels are facing toward each other.



2

Gently place the second ion reservoir stack (cathode stack) on the gel.



Use the blot roller to remove any air bubbles in the assembled transfer pack and provide consistent contact between the layers.

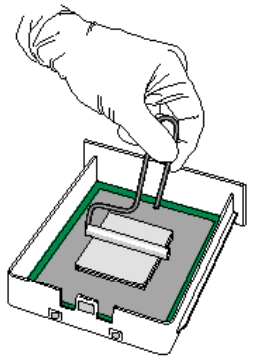


Fig. 10. Assembling the mini format transfer pack.

Table 3. Bio-Rad preprogrammed protocols.

Protocol Name	MW, kD	Time, min	2 Mini Format Gels or 1 Midi Format Gel (per cassette)	1 Mini Format Gel (per cassette)
Standard SD	Any	30	Up to 1.0 A; 25 V	
1.5 mm GEL	Any	10	2.5 A, up to 25 V	1.3 A, up to 25 V
High MW	>150	10		
Low MW	<30	5		
Mixed MW (Turbo)	5–150	7		
1 Mini-TGX	5–150	3	–	2.5 A, up to 25 V

- With long transfer times or high power conditions, some very low molecular weight proteins may transfer through the membrane to the lower ion reservoir stack. Use a shorter transfer time or reduce power conditions for the most efficient transfers

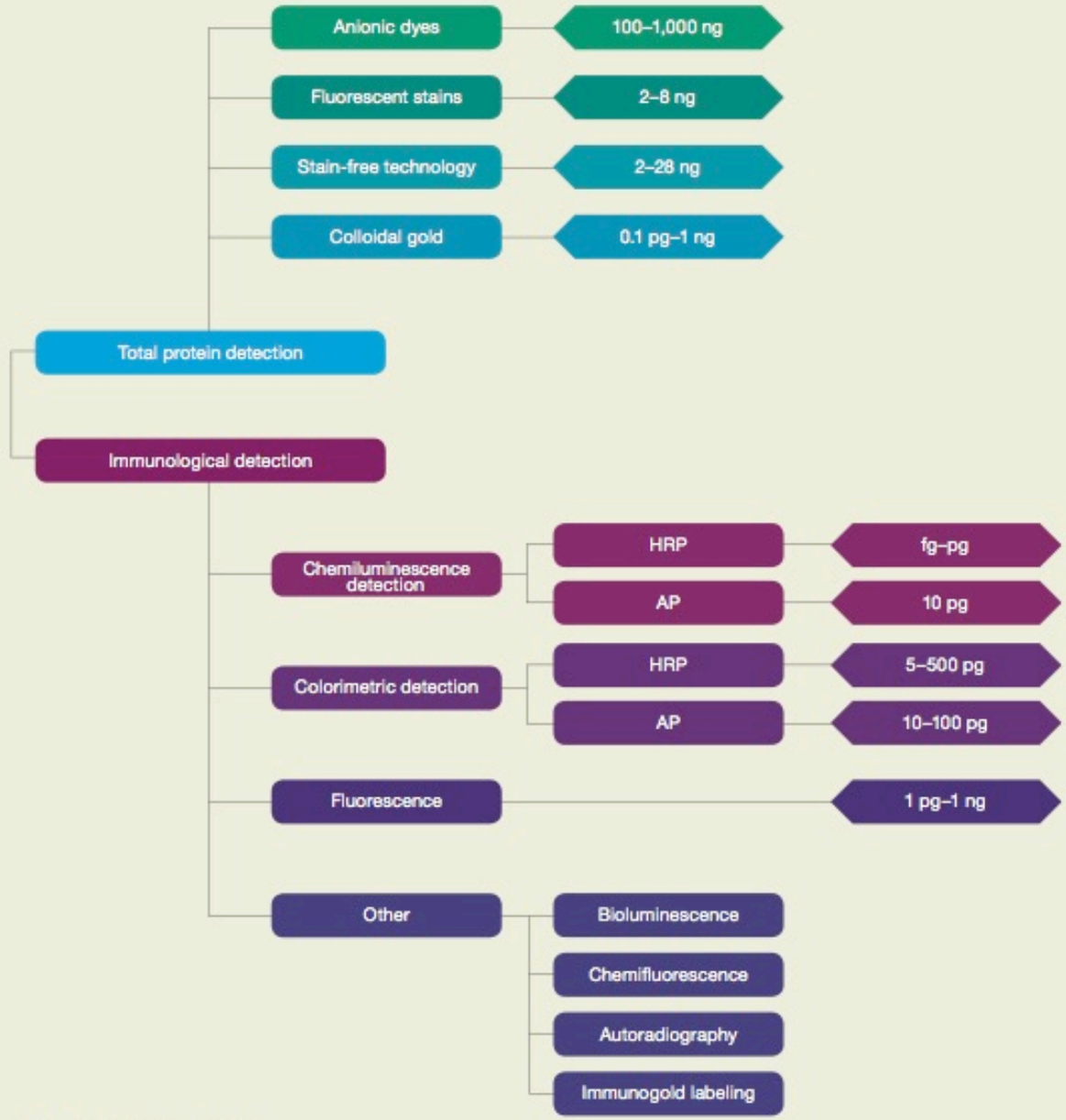


Fig. 5.1. Protein detection systems.