PROTEIN ISOLATION FROM FFPE TISSUES

From blank sections of formalin-fixed and paraffin-embedded tissue. The extracts can be used for western blot or protein arrays.

After deparaffinization, the sections are incubated in a extraction buffer at two different T to render reversible formalin crosslinks therefore allowing protein isolation.

STEPS

- Sections cut- max 3 sections 10 µm for a 100 mm^2 area.
- Dewaxing and 3 EtOH washes: 100, 96 o 90%, 70%

Estraction

100 µl buffer

1. *100°C for 0 min.*
2. *80°C for 2 h shaking.*
3. *4°C for 1 min .*
4. *Centrifuge for 15 min @ 14,000 x g at 4°C. Collect spn with proteins.*

Troubles

Bassa resa

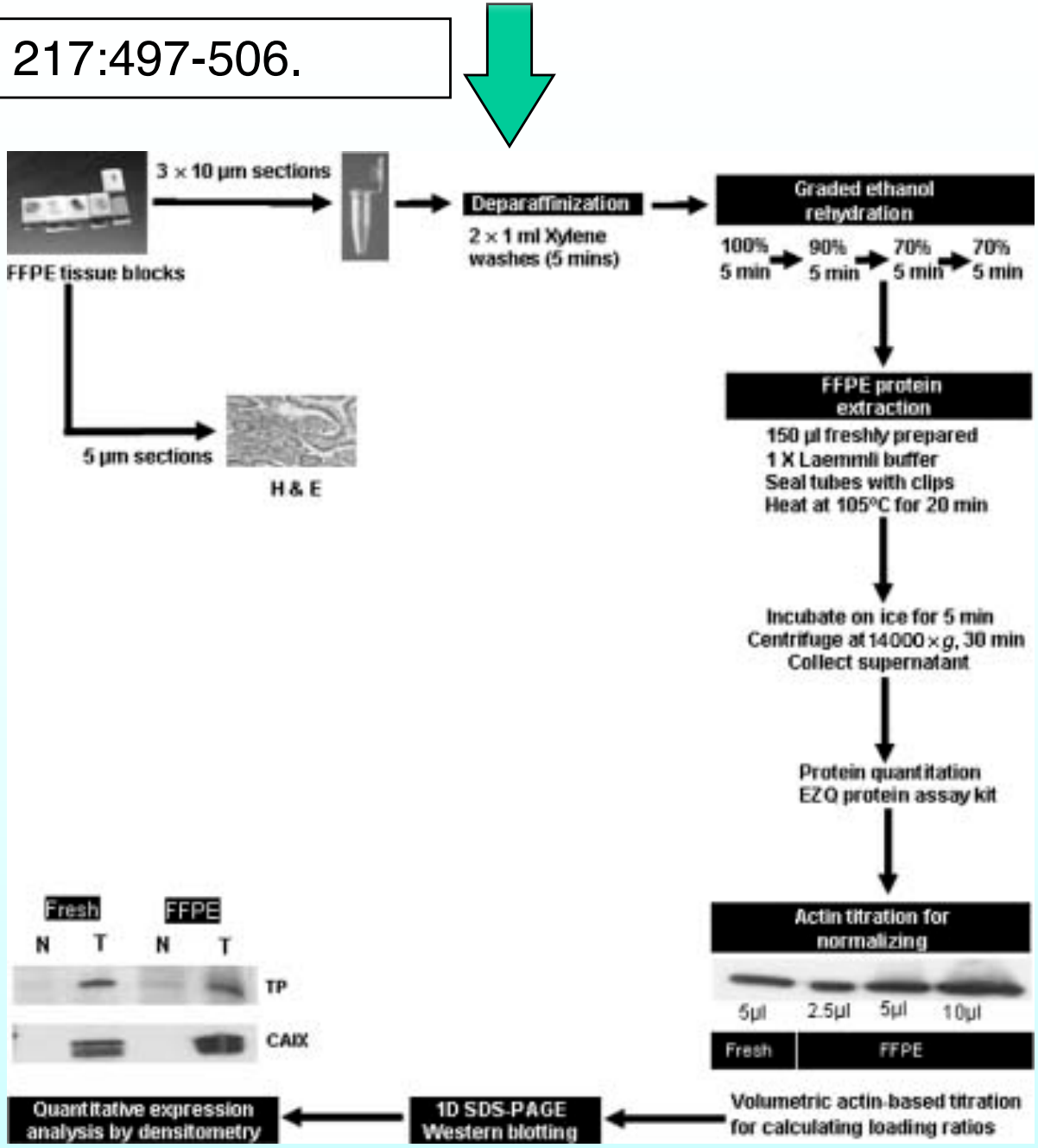
a) Low yield: tissue fixed longer than 24 h or stored very long time can lead to incomplete extraction

b) Little starting materials

c) Incomplete de-waxing or too much paraffin in teh sample. Difficult removal from thick sections.

PROTEIN ISOLATION FROM FFPE TISSUES

J Pathol 2009; 217:497-506.



STEPS

➪ Sections cut- max 3 sections 10 µm for a 100 mm^2 area.

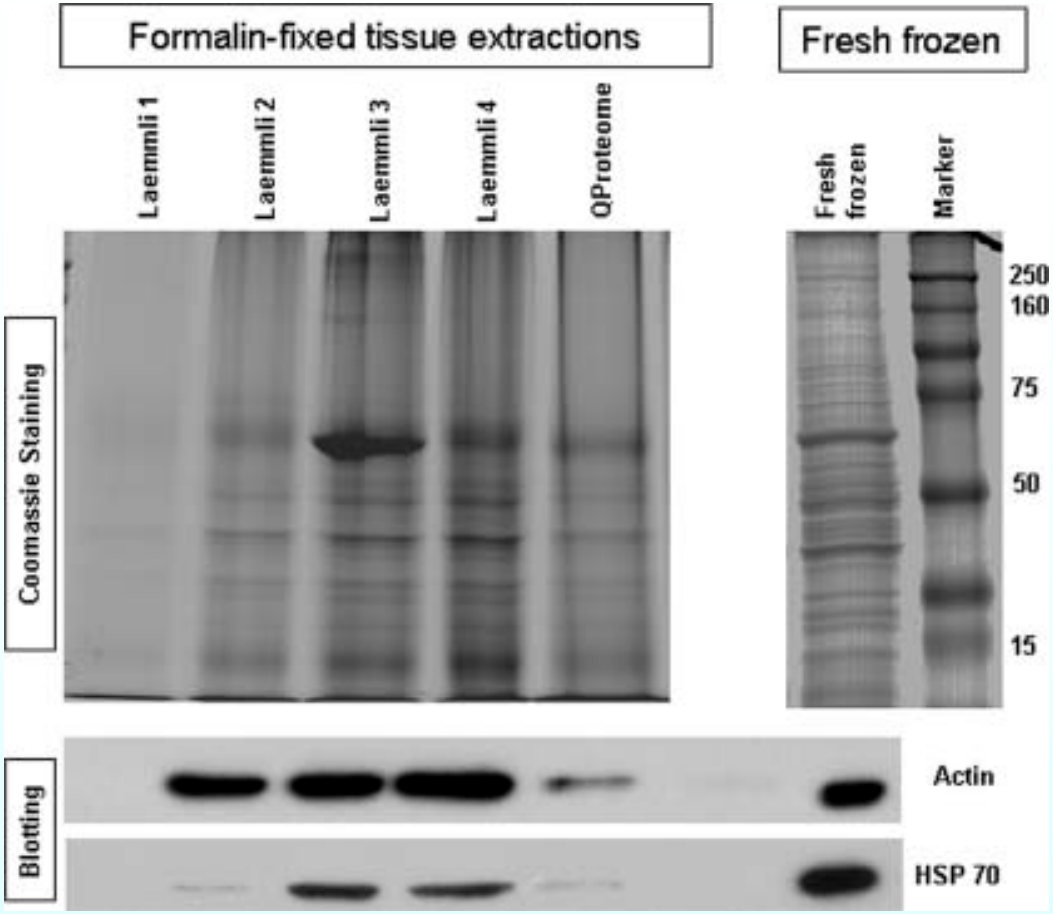
➪ Dewaxing: xylene (1 ml for 5 min, 2 x) and 3 EtOH washes: 100, 90 e 70%, 5 min each

Isolation step

150 µl Laemmli buffer: 100 mM Tris-HCl, pH 6.8; 2% (w/v) SDS; 20% (v/v) glycerol; 4% (v/v) ß-mercaptoethanol (add just before use)

1. *105°C for 20 min.*
2. *Ice for 5 min .*
3. *Centrifuge for 30 min @ 14,000 x g at 4°C. Collect spn with proteins. Store @ -20° C.*

Comparison Qproteome- Laemmli



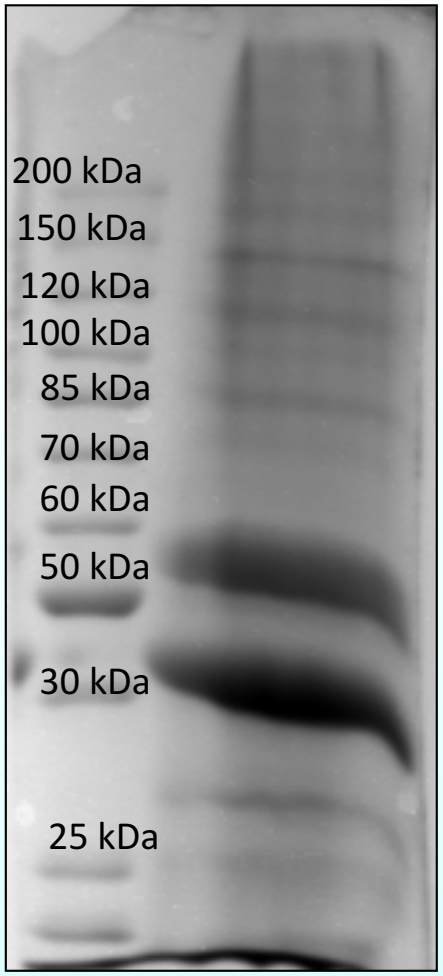
Laemmli 1: no boiling; **Laemmli 2:** 105° C 10 min; **Laemmli 3:** 105° C 20 min; **Laemmli 4:** 105° C 20 min, 80° C 20 min; **QProteome:** 100° C 20 min, 80° C 2h.

Protein isolation from alchol based fixatives (RCL-2; FineFix) modified protocol*

- **3 x10 μm blank sections**
- **Dewaxing** (2x xylene, 1 ethanol 100%, 1 ethanol 96%, 1 70%)
- Disloge the pellet in **100 μl buffer** (50 mM Tris-HCl pH7.5, 7M UREA, 2M THIOUREA, 2% CHAPS, 1% MEGA, 0.5% TRITON X-100, 1% OGP AND 50 MM DTT) including protase inhibitors
- **vortex**
- **15' ice + 20' 99° C + 1h 80°** C
- **Centrifuge** @ max rate for 15min @4° C

V Bellet , F Boissière, F Bibeau, C Desmetz, ML Berthe et al. **Proteomic analysis of RCL2 paraffin-embedded tissues. J Cell Mol Med** 2007 Dec 10. [Epub ahead of print]

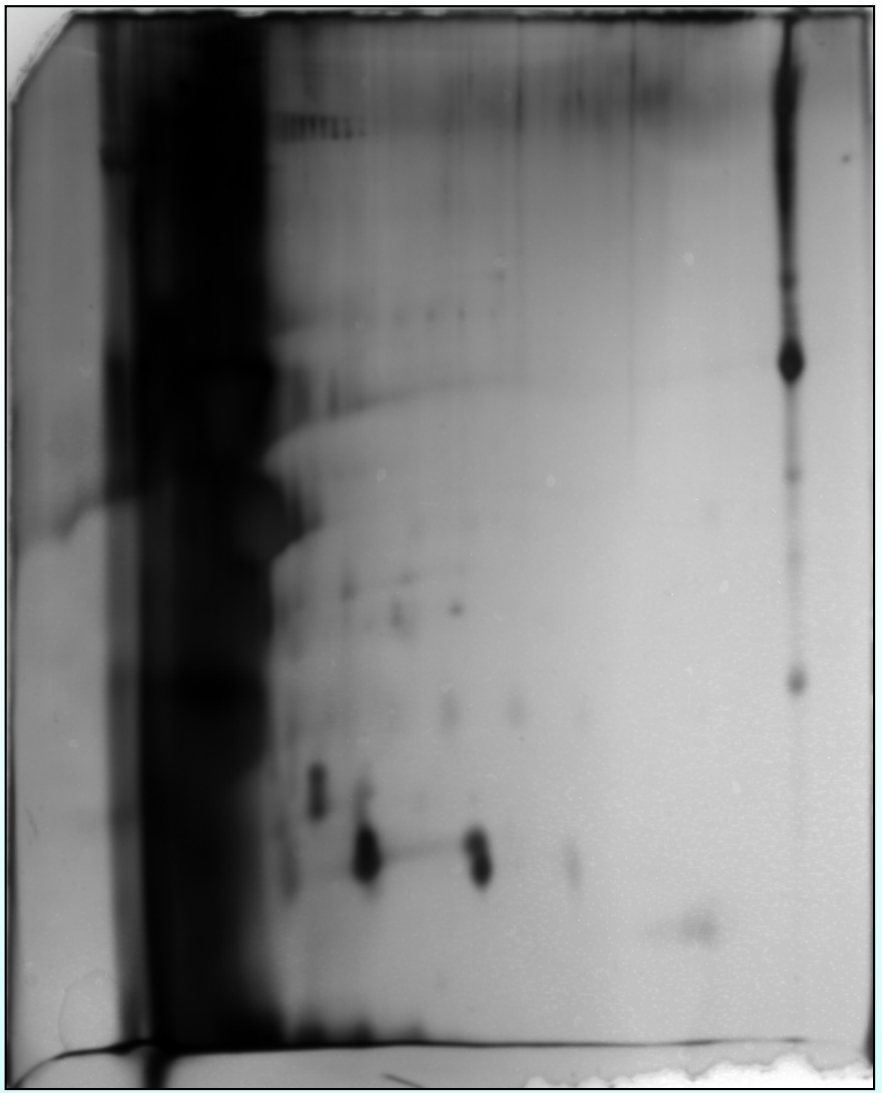
15 ul per lane



Coomassie Staining

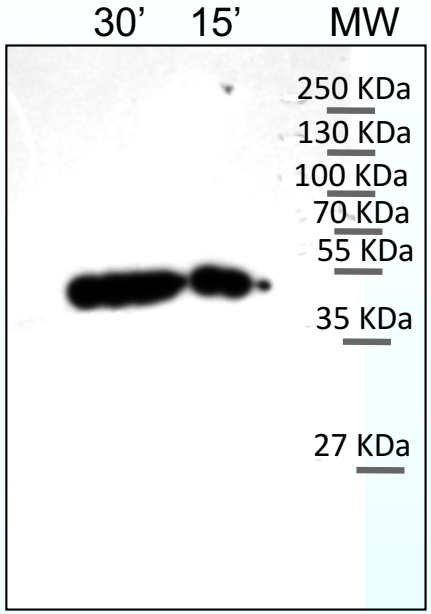
50 ul loaded

\+ pH 3-10 NL, strip 7 cm -



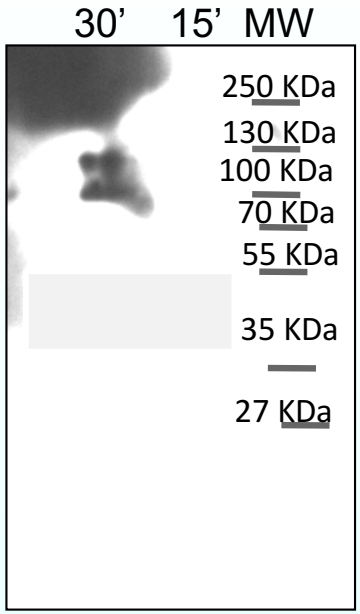
Silver Staining

Western blot analyses



β-actin (42kDa)

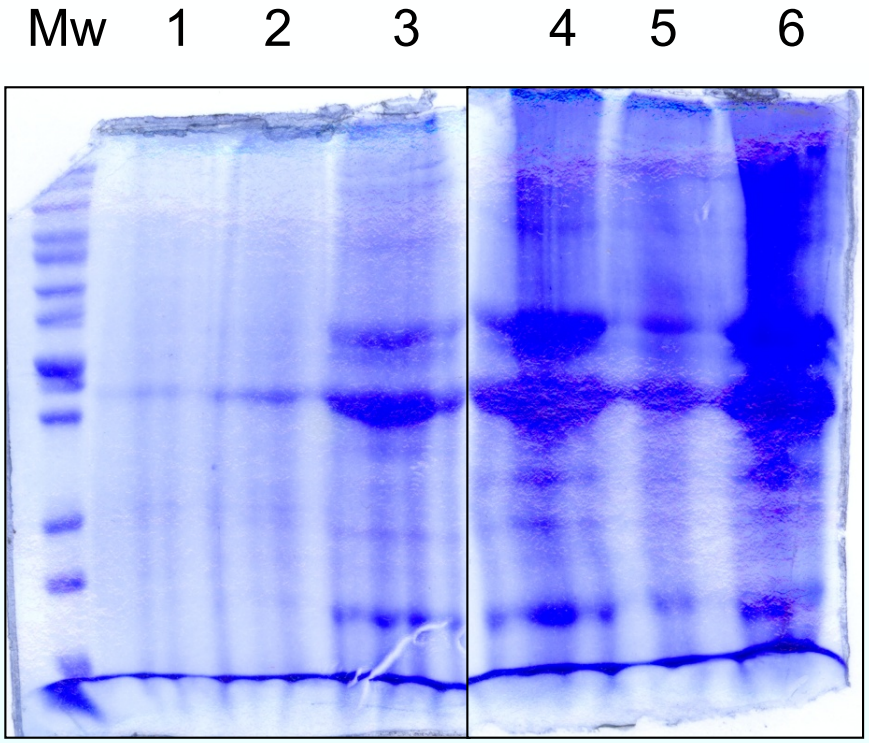
5” exposure



HER2(182kDa)

10” exposure

Note: 30’: after 15’ in ice, the sample was boiled for 30’
15’: after 15’ in ice, the sample was boiled for 15’



1 #6666 DW FX ISOPROPANOLO (20’ 99° C)

2 #6557 BL28 FXD ISOPROPANOLO (20’ 99° C)

3 #12205 BL14 FX JFC (20’ 99° C)

4 #12776 FX JFC (20’ 99° C)

5 #8467 RCL2 4C (15’ ice + 20’ 99° C)

6 #12776 FX JFC (15’ ICE + 20’ 99° C + 1h 80° C)