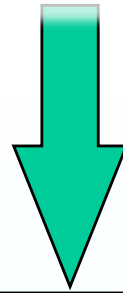


PROTEIN ISOLATION FROM FFPE TISSUES



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 an 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.

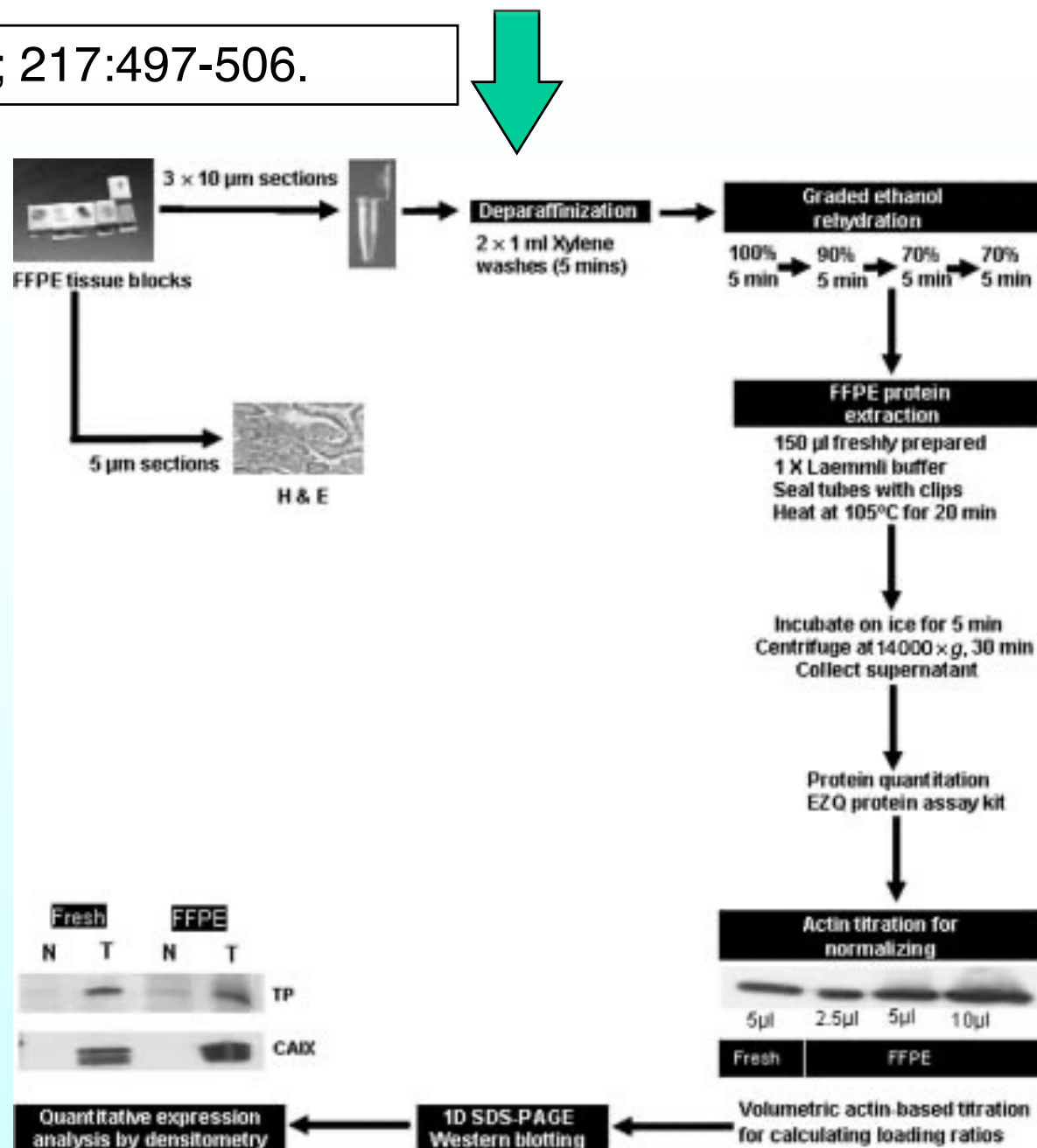
Troubles

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 the 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

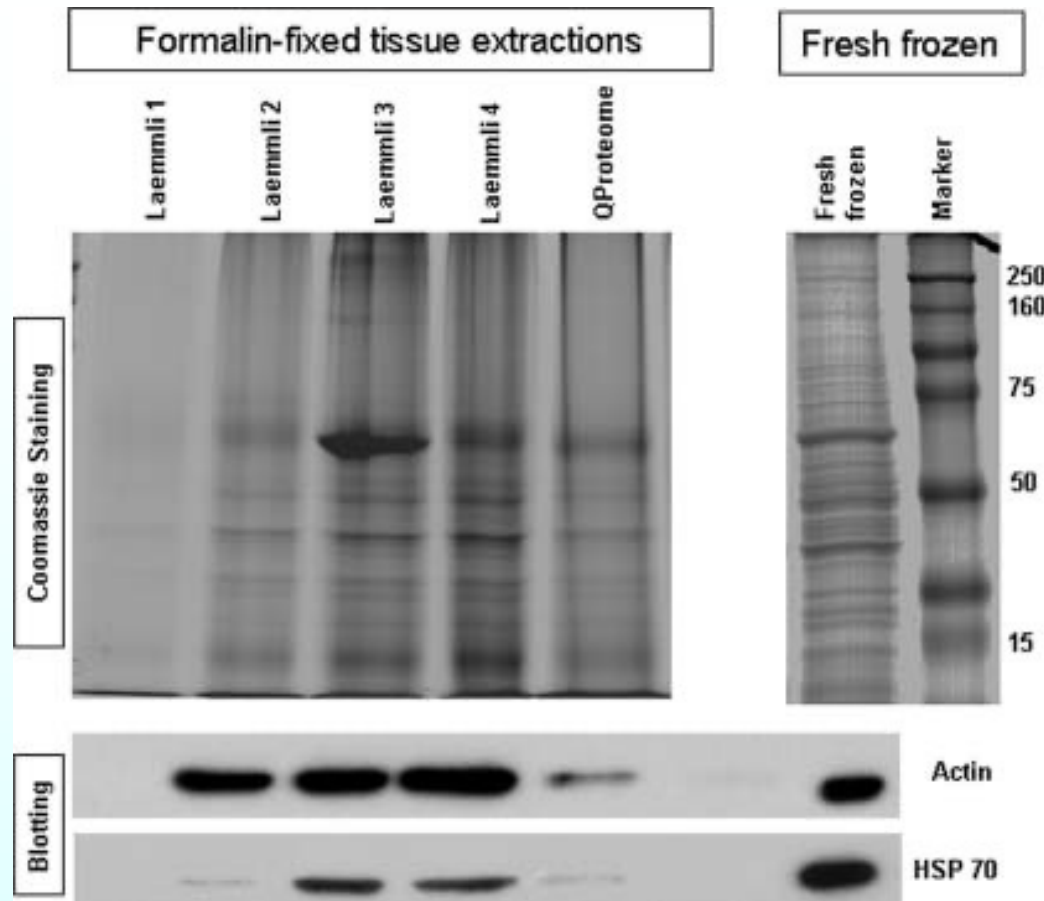
Isolation step



150 μl Laemmli buffer: 100 mM Tris-HCl, pH 6.8; 2% (w/v) SDS; 20% (v/v) glycerol; 4% (v/v) β -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.

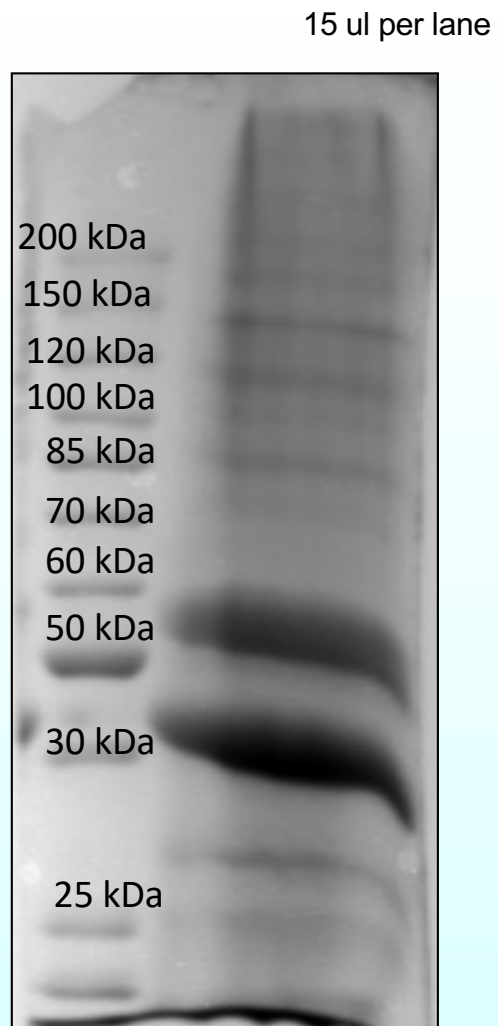
Comparison Qproteome- Laemmli



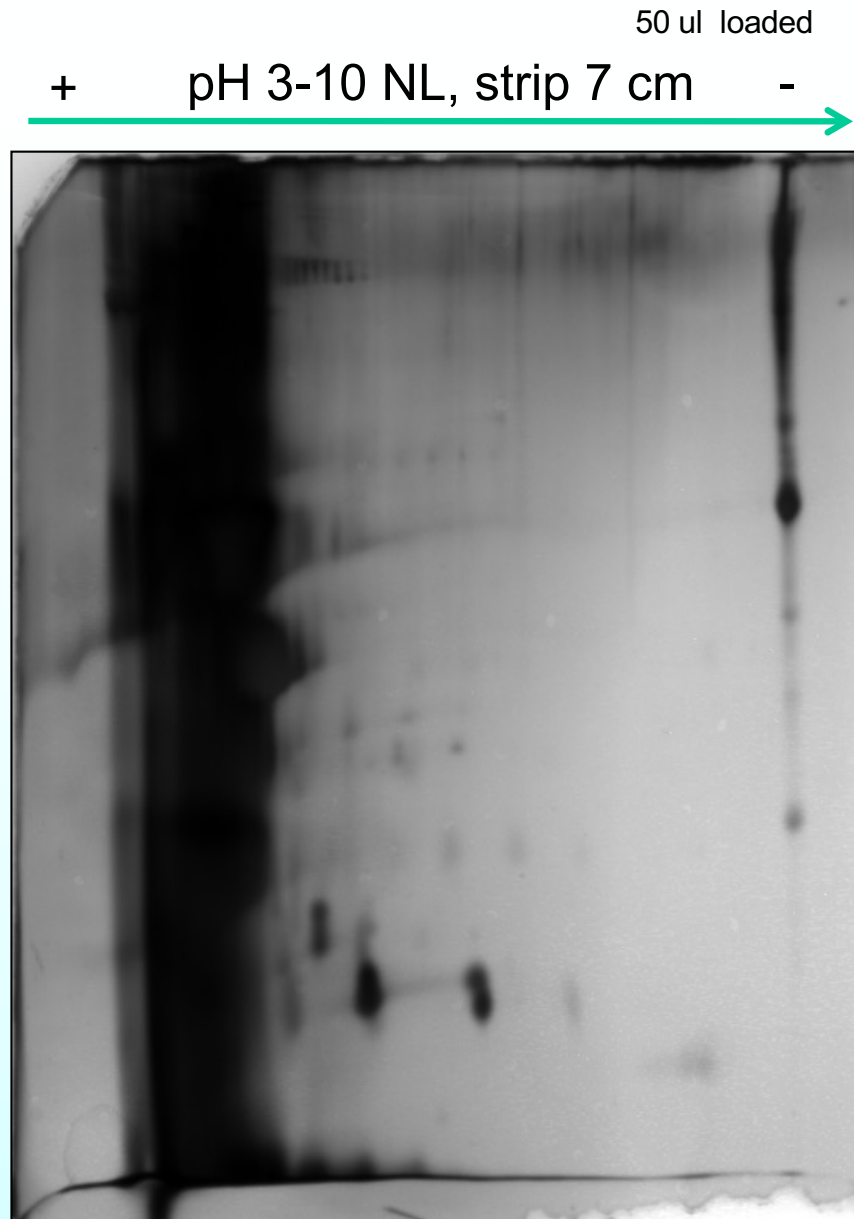
Laemmli 1: no boiling; **Laemmli 2:** 105° C 10 min; **Laemmli 3:** 105° C 20 min; **Laemmli 4:** 105° C 20 min, 80° C 20 min; **QProteome:** 100° C 20 min, 80° C 2h.

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

- **3 x10 μ m blank sections**
- **Dewaxing** (2x xylene, 1 ethanol 100%, 1 ethanol 96%, 1 70%)
- Dislodge the pellet in **100 μ l 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 protease inhibitors
- **vortex**
- **15' ice + 20' 99° C + 1h 80° C**
- **Centrifuge @ max rate for 15min @4° C**

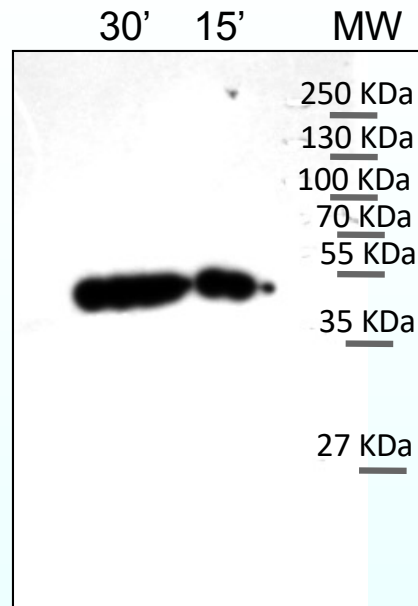


Coomassie Staining



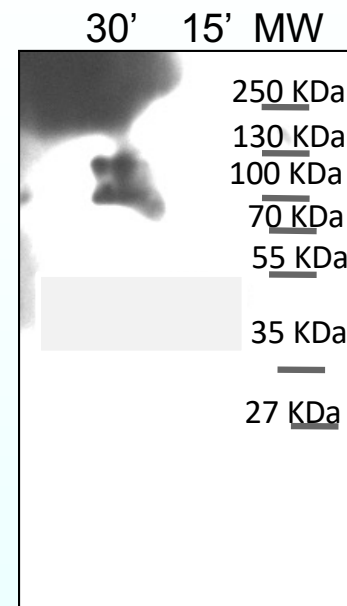
Silver Staining

Western blot analyses



β -actin (42kDa)

5" exposure

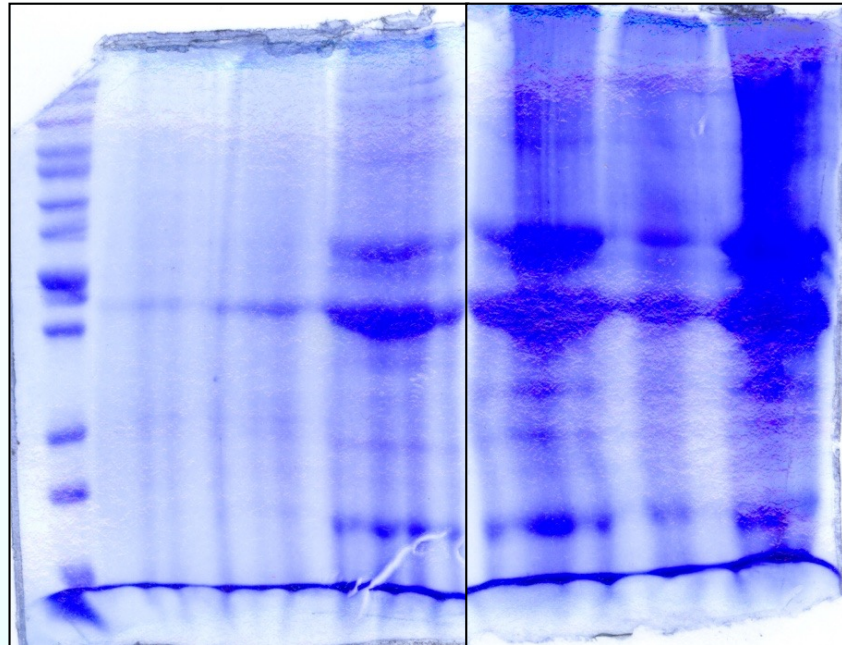


HER2(182kDa)

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'

Mw 1 2 3 4 5 6



- 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)