Coomassie Blue Staining Methods in the Staining Trays: -normal, large and –Multi 6-

EDC V08/17

Fig.1: staining tray normal+ lid

Fig 2: staining tray large + lid

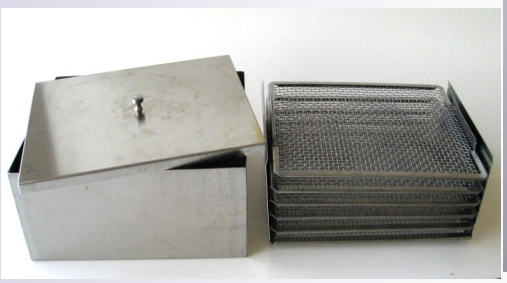


Fig 3: staining tray large multi6

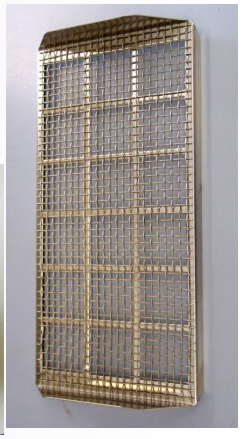


Fig.5: grid(s) inside

The Staining Trays:

staining tray standard size (+ grid + lid) *edc-wm-n1*
For normal gels (150 × 300 × 50 mm), working volume 600 mL, see figure 1
staining tray large size (+ grid + lid) *edc-wm-l1*
For large gels (220 × 280 × 60 mm), working volume 1.2 L see figure 2
staining tray multi6 (+ 6 grids + lid) *edc-wm-m6*
For 6 large gels (220 × 280 × 60 mm), working volume 6 L see figure 3

Additionally:

Coomassie R350 tablets Aldrich B4921-20TAB

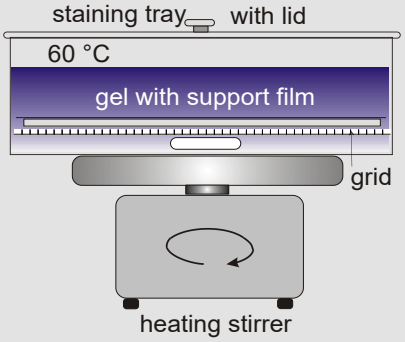


Fig.6: „Hot Coomassie Staining"

Hot Coomassie Staining

This hot Coomassie-staining is staining and fixing at the same time (see figure 6). The acetic acid for staining and destaining can be of technical quality.

Attention: Do not heat NF-film supported gels to 60°C because they are heat sensitive! Max temperature for NF-film supported products 40°C: All proteomic gels are also available on normal films.

For 2D-gels: Unfortunately standard IPG buffers and Pharmalytes can cause a partly dark background.

Stock solutions: (Volumes for standard size gels, 12 × 26 cm, use double volume for large gels)

staining solution: 0.03 % (w/v) Coomassie R-350 (2 tablets) in 2.5 L 12.5% acetic acid. Pour 600 mL into the staining tray standard size; staining tray large size: 1.2 L; multi6: 6 L. Stir while heating.

destaining solution: 200 mL 12.5% (v/v) acetic acid

preserving solution: 200 mL ~5% (v/v) glycerol

Staining programme: *fresh* staining solution at 60 °C (in a fume hood) while stirring.
200 mL destaining solution in a tray on a rocking platform.
200 mL impregnating solution in a tray on a rocking platform.

Optimal destaining: Can be achieved when the gel is placed in a small volume (150 mL) destaining solution after the staining process, overnight.

Quick Hot Staining 60°C —gel thickness: 0.4 to 0.6 mm— —gel thickness 0.6 to 1.0 mm—

Step	Standard gels	ElphGel 2D	Standard gels	ElphopGel 2D
Staining (0.03% CBB-R350, 12.5% HAc)	30 min	45 min	1 h	1 h
Destaining (12.5% HAc)	3 x 20 min	overnight	3 x 30 min	overnight
Preserving (~5% Glycerol)	30 min	30 min	45 min	1 h

Drying: air-dry overnight

Colloidal Coomassie Staining

(Attention: use the normal film supported gels, NF films will not sustain more than 30% ethanol!). For staining of proteomic gels we suggest to do the „semi colloidal hot Coomassie" staining, next method.

Stock solutions:

A) Coomassie Brillant Blue G-250 Soluting (**CBB**-stock)
(5 g Coomassie Brillant Blue G-250, add 100mL H_2O_{dist}*)*

B) Colloidal Coomassie Staining Solution (**CCD**-stock)
(50 g $(NH_4)_2SO_4$ *+ 6 mL 85% (w/v) Phosphoric acid, fill up to 490mL with* H_2O_{dist} *add 10ml CBB-stock*)

C) The final Colloidal Coomassie Solution (**CCS**-solution)
(200 mL CCD-stock + 50 mL MeOH),
Volume is given for standard size gels, for large gels: double this volume.
Prepare the Colloidal Coomassie Solution always freshly!

Protocol: (200 mL: standard size gel, 400 mL: large gel)

2 h fixing in 40% Ethanol (EtOH), 10% Acedic acid (HAc)
2 × 10 min washing in H_2O_{dist}
overnight (15 h) staining in Colloidal Coomassie Solution (CCS)
2 × 15 min washing #1 in 20% methanol (MeOH)
2 × 1 h washing #2 with H_2O_{dist}

If the gel have to be dried: 20 min 10% Glycerol, air dry 2 h, then put it back in the bag.

Colloidal Coomassie Staining	gel thickness: 0.4 to 0.6 mm	gel thickness: 0.65 mm to 1.0 mm
step	**all matrices**	**all matrices**
Fixing (40% EtOH, 10% HAc)	2 h	2 h
Washing (H_2O_{dist})	2 × 10 min	2 × 10 min
Staining (CCS)	Overnight (15 h)	Overnight (15 h)
Washing #1 (20% MeOH)	2 × 10 min	2 × 15 min
Washing #2 (H_2O_{dist})	2 × 30 min	2 × 1 h

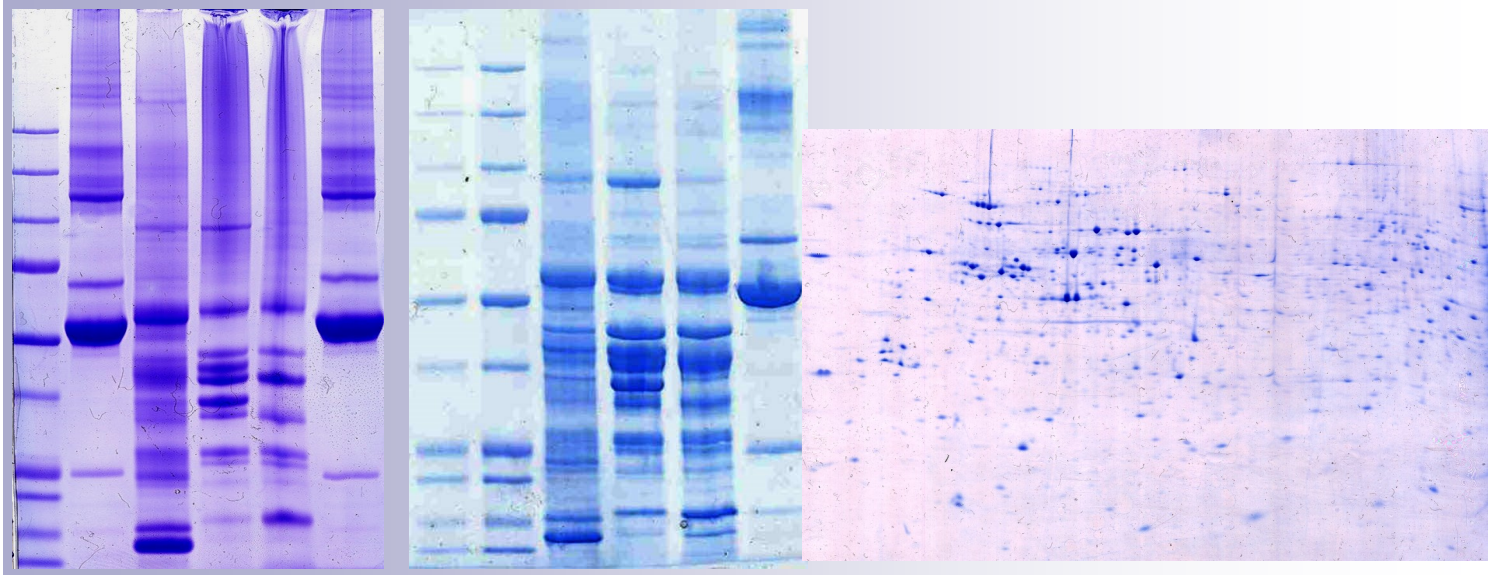


Fig.6: Quick Hot Coomassie (left) and Colloidal Coomassie Staining (center and right)

Semi-Colloidal Coomassie Staining

The semi colloidal Coomassie-staining is staining and fixing at the same time (see figure 6). The Phosphoric acid is less lipophilic than the Acetic acid. This results in a slightly colloidal staining. The 3% Acetic Acid is only used for getting the Coomassie better solubilized and we achieve therefor a „tank-clean" staining recipe. We recommend this method for peptides and preparative 2D-gels.
Attention: Do not heat NF-film supported gels to 60°C because they are heat sensitive! Max temperature for NF-film supported products 40°C: All proteomic gels are also available on normal films.

The chemicals for staining and destaining can be of technical quality.
Attention: The non-fluorescent film supports are heat-sensitive: All proteomic gels are available also on normal films.
When the hot fixing is used: We recommend to use the IPG Ampholytes Mix (ETC 1004-15) for rehydration of IPG strips, because standard IPG buffers and Pharmalytes can cause a partly dark background.

Stock solutions: (Volumes are given for normal sized gels, 12 × 26 cm)
staining solution: 0.01 % (w/v) Coomassie R 350 (1 tablet) in 2.8 L H_2O_{dist}
+ 0.5% (w/v) Phosphoric acid (18 mL) + 3% HAc (90 ml HAc) —> fill up to 3 L

destaining solutiont: 200 mL 10% HAc; 400 mL for large gels.
preserving solution: 200 mL 10% (v/v) glycerol; 400 mL for large gels.

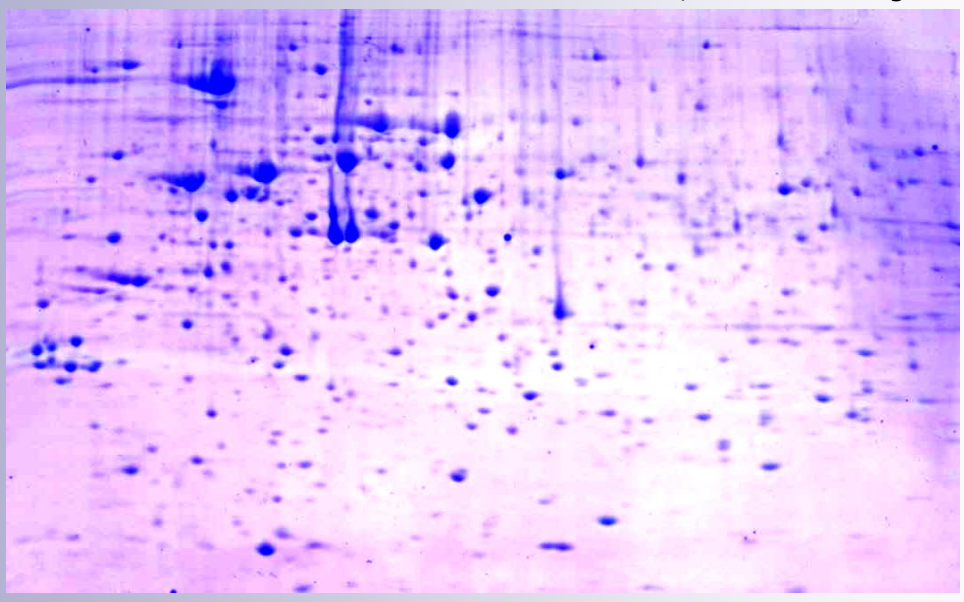
Staining programme: *fresh* staining solution at 60 °C / 40°C***** *(while strongly stirring, see figure 6), ,* destaining *(in a tray on a rocking platform), If the gel has to be dried: 1 h in 10 % glycerol, air dry 2 hours, then put it back in the bag.*

Semi-Colloidal Coom. ----gel thickness: 0.4 to 1.0 mm-------

step	standard matrix	2DGel flatbed matrix
Staining* (see above)	1-2 h (or overnight)	2 h - overnight
Destaining (10% HAc)	2 × 20 min	2 × 30 min
Preserving (10% Glycerol)	30 min	40 min

***** normal gel-support: **60°C /** NF-film supported gels: max **40°**

Second dimension:Semi-colloidal Hot Coomassie, stained overnight



ElphGel 2D large 12.5%