

# SAMPLE PREPARATION GUIDE

## READ BEFORE SAMPLE PREPARATION

Samples not compliant with this guide will be slower in the platform process.

EDyP-service platform does not accept infectious or radioactive samples

### SUMMARY:

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## 1) PRECAUTIONS TO TAKE DURING SAMPLE HANDLING

PRINCIPAL CONTAMINANT IN PROTEOMIC ANALYSES = KÉRATINES (HUMAN ORIGIN: HAIR AND/OR ANIMAL ORIGIN: GARMENTS CONTAINING WOOL)

PROBLEM: CONTAMINATION MASKING THE PROTEIN OF INTEREST IN YOUR SAMPLE

### SOLUTIONS:

#### FOR PERSONS HANDLING THE SAMPLES:

- Wear a clean lab-coat that completely covers your arms.
- Avoid wearing woollen garments.
- Wear gloves at all times during sample treatment, from electrophoresis gel preparation to sending samples to the platform. After putting on gloves, wash them with hot soapy water (*glove boxes are a source of keratins!*), rinse them abundantly and avoid touching anything but the instruments to cut your gel (telephone etc.)
- Tie back long hair
- Avoid leaning your body/head and do not discuss over your sample!

#### FOR LABORATORY MATERIAL:

- Wash lab bench where sample will be prepared in order to avoid dust.
- Wash all equipment that will be in contact with the sample with hot soapy water (tweezers, electrophoresis material, transparency on the scanner glass, transparency under the gel during cutting etc.). Rinse with clear water.
- During staining/destaining steps for electrophoresis gels, use clean cone boxes. The boxes should not be the same ones that are used for Western-Blot experiments for example. (This will help to avoid contamination by proteins used to saturate membranes such as casein or albumin.)
- Use fresh buffer/solutions in order to avoid protein contaminations linked to bacterial development or mould.

The quality of your results is directly linked to the quality of your preparation!!!

## 2) SAMPLE MIGRATION ON 1D GEL ELECTROPHORESIS

For the electrophoresis step, precast gels are recommended (NuPAGE for example) as well as commercial buffers in order to reduce any risk of contamination. In any case, buffers should be prepared freshly and used with the appropriate precautions. Water used to prepare buffers should be taken freshly from an ultra-pure water production station (e.g. Elga or Millipore; 18,2 MΩ) or from a commercial (HPLC grade) source.

The migration protocol described below concerns NuPAGE gels.

### SOLUTIONS TO PREPARE:

- **Tris 0.5M pH 6.8** solution: weigh 6.05 g of tris base and add 50 mL Ultrapure water. Adjust pH to 6.8 with HCl and complete to 100mL with Ultrapure water (the solution should be stored at 4°C for no longer than 6 months).
- **Laemmli 5X** buffer: mix 5mL of Tris/HCl 0.5M pH 6.8, 2 g of SDS, 4 g of glycerol, 5mL of β-mercaptoethanol, 1.25mL of 1% bromophenol blue, complete to 20mL with Ultrapure water. Aliquot buffer (1 mL per aliquot) and store at -20°C for no longer than one year.
- **NuPage MOPS SDS running buffer 20X**: 104.6g of MOPS 1M, 60.6g of Tris Base 1M, 10g of 2% SDS and 3g of EDTA 20mM, qsp 500mL of Ultrapure water. The final solution should be at pH 7.7 (do not adjust the pH). Store at 4°C for up to 6 months.
- OR NuPage MES SDS running buffer 20X**: 97.6g of MES 1M, 60.6g of Tris Base 1M, 10g of 2% SDS and 3g of EDTA 20mM, qsp 500mL with Ultrapure water. The final solution should have a pH of 7.3 on a 1X dilute aliquot.(do not adjust). Store at 4°C for up to 6 months.
- **NuPage running buffer 1X**: add 50mL of NuPage SDS running buffer 20X (MOPS or MES) to 950mL of deionized water.
- **Cathode Buffer**: add 500μL of NuPage Antioxidant to 200mL of NuPage SDS running buffer 1X.

### SAMPLE PREPARATION:

- Mix 4 volumes of protein sample with 1 volume of Laemmli 5X buffer.
- Heat with a heater block for 10 min at 95°C to denature proteins.
- Centrifuge rapidly (maximum speed on a bench centrifuge for 1 min).

### SAMPLE MIGRATION:

- Prepare the migration system and deposit the sample according to manufacturer's instructions.
- Migrate sample as needed:

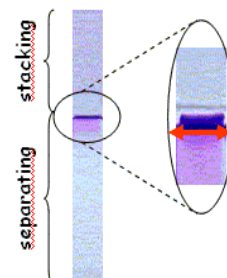
#### "Stacking" migration

Migrate the gel at room temperature at 200V/125 mA. Stop migration when the sample entirely entered the gel (1 to 3 mm separation) (just under wells in the case of precast gels; after the stacking gel in case of home-made gels).

NuPage gel:

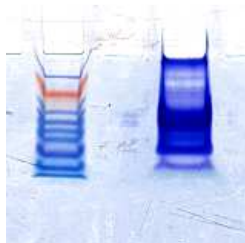


home-made gel:



"Pseudo-separating" migration

Migrate the gel at room temperature at 200V/125 mA. Use coloured molecular weight markers to help decide when to stop the migration.



"Complete separating" migration

Migrate the gel at room temperature at 200V/125 mA. Stop migration when bromophenol blue reaches the bottom of the gel.



### 3) GEL ELECTROPHORESIS STAINING AND UNSTAINING PROTOCOLS COMPATIBLE WITH MASS SPECTROMETRY

#### COOMASSIE BLUE STAINING:

At the end of the migration, the gel is unmolded and placed for 1 h in a solution of Coomassie Blue (e.g. Coomassie Brilliant Blue R250 stain: Biorad 161-0436, do not reuse the solution).

Destain the gel with several destaining buffer baths: acetic acid/ethanol/milli-Q water (7.5/30/62.5) (v/v/v).

#### SILVER NITRATE STAINING:

At the end of the migration, the gel is unmolded and fixed for 1 h in fixing buffer: acetic acid/ethanol/milli-Q water (7.5/30/62.5) (v/v/v).

The staining is done by successive baths of 100 mL as described below:

#### Solutions to prepare extemporaneously:

- Sodium Hydrosulfite  $\text{Na}_2\text{S}_2\text{O}_4$  0.3 g/L: weigh 30 mg for 100mL of milli-Q water
- Silver Nitrate 2 g/L: weight 200 mg for 100mL of milli-Q water
- Solution A:
 

$\text{K}_2\text{CO}_3$	3 g
HCHO	25 $\mu\text{L}$
$\text{Na}_2\text{S}_2\text{O}_3, 5\text{H}_2\text{O}$	100 $\mu\text{L}$ of a solution at 10 mg/mL (1mg)
Milli-Q $\text{H}_2\text{O}_2$	QS 100 mL
- Tris/acetic acid: weigh 40 g of Tris, solubilise with 500mL of milli-Q water, add 20mL of acetic acid and complete to 1 L with milli-Q water.

#### Protocol:

- Rinse with 10% ethanol in milli-Q water for 20 min; eliminate the solution and repeat this step.
- Rinse with milli-Q water for 20 min; eliminate the solution and repeat this step.
- Sensitisation: Place gel in  $\text{Na}_2\text{S}_2\text{O}_4$  for 1min, eliminate the solution by pouring.
- Rinse with milli-Q water for 1 min; eliminate the solution and repeat this step.
- Stain with a bath of Silver nitrate for between 20 and 60 min; eliminate the solution.
- Rinse rapidly (10 to 20s) with two milli-Q water baths.
- Reveal with solution A for between a few minutes and one hour depending on the concentration of proteins (stop revelation when the bands/spots appear. If there is too much contrast, the bands/spots will be too large).
- Stop staining with Tris/acetic acid for a minimum of 30 min.

**Caution:** Anticipate time for the destaining and cutting of bands (or spots) just after staining in case of silver staining. In the case of a proteomic analysis: the silver stained gels should be unstained ASAP.

In fact, silver nitrate coloration of proteins in gels induces reticulation of Lysine residues. This affects the different steps of sample preparation (incomplete trypsin cleavage after K and extraction of the peptides out of the gel - Rabilloud *et al*, 2007). This is true even for protocols "compatible with mass spectrometry". For these reasons, it is MANDATORY to unstain the pieces of gels just after the silver staining operation.

#### Solutions to prepare:

- Stock solution of ammonium bicarbonate 1 M ( $\text{NH}_4\text{HCO}_3$ ): 11.9 g of ammonium bicarbonate in 150 ml of milli-Q water.
- Solution A: Ammonium bicarbonate 25 mM. Dilute 2.5 mL of stock solution of ammonium bicarbonate 1M in 97.5 mL milli-Q water.
- Solution B: 25 mM ammonium bicarbonate and 50% acetonitrile. Dilute 2.5 mL of Ammonium bicarbonate 1 M in 47.5 mL of milli-Q water and add 50 mL of acetonitrile.

- Hydrogen Peroxide Solution 7 % final in milli-Q water.
- All these solutions should be prepared freshly.

**Protocol:**

Band unstaining involves successive washing steps with 200  $\mu$ l of the different buffers. All steps should be carried out under agitation and in this order:

- Solution B for 30 min; eliminate solution
- Solution A for 30 min; eliminate solution
- Solution B for 30 min; eliminate solution
- Solution A for 30 min; eliminate solution
- Milli-Q water for 15 min; eliminate solution
- 100% Acetonitrile for 15 min; eliminate solution
- Eliminate the supernatant with a pipette and dry the pieces of gel in a Speed-vac for approximately 5'.
- Hydrogen peroxide ( $H_2O_2$ ) 7% (v/v) for 15 min; eliminate solution
- Milli-Q water for 15 min; eliminate solution
- 100% Acetonitrile for 15 min; eliminate solution
- Milli-Q water for 15 min; eliminate solution
- Stock at  $-20^\circ C$
- Send samples in dry ice

#### 4) GEL BAND EXCISION

Do not forget to wash the cutting support with hot soapy water and to rinse it with milli-Q water afterward.

Cut gel pieces precisely; excess of polyacrylamide is not beneficial for the analysis.

Due to precise calibration of the sample preparation robot on the EDyP-Service platform, it is strongly advised that the final volume of the gel pieces is around 1.5 mm<sup>3</sup>.

##### CUTTING BANDS FROM 1D GELS:

1D gel bands should be cut with a scalpel. The cut band should be divided into three or four cubes of about 1 mm<sup>3</sup>.

Place the pieces of pieces of gel into the Eppendorf tube or into the corresponding microplate well filled with destaining buffer.

##### CUTTING SPOTS FROM 2D GELS:

2D spots can be cut with 2 or 3 mm diameter Biopsy Punches. After having deposited the gel on the support, remove the gel piece by stabbing frankly with the Biopsy Punch without rotary movement. Incline the tool in order to keep the piece of gel inside and let it drop into the vial or corresponding well of the plate fill with staining/destaining buffer; use a clean capillary or cone if needed to make it drop.

## 5) SENDING SAMPLES

When you have filled your tubes or plates with your samples:

- Ensure water tightness; close the tube lid or seal the microplate; put some Parafilm on tubes that have do not have "safe-lock" caps.
- Fill in the corresponding sample datasheet: this is important for the identification and traceability of your samples (ask the platform if you need more explanations)
- Place the sample and the sample datasheet in a padded envelope or a box addressed to the platform - Express post is preferred - labelled with this address:

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