Protocol Coomassie Staining and

Storage of SDS-PAGE Gels for Proteomics

- *Treat gels carefully - don't hold your bare hands/arms/face above an open container with your gel, in order to minimise contamination with keratin proteins. Keratins are found in hair, skin particles, dust, wool sweaters, etc., are readily detected in the mass spectrometer, and can interfere with your analysis.*
- *Use a clean container with tight-fitting lid [NEVER used before for, e.g., western blot blocking with BSA, low fat milk, etc; LOW polymer release: household storage containers made from polypropylene are OK].*
- *Instead of decanting, you can use a 25-ml pipette during solution changes. Always wear gloves, and stay away from the open container with the rest of your body.*

Staining Procedure

1. Transfer gel to a clean container with **Fixing Solution**. Rock on a platform for 10-20 min.
   Incubation time depends on gel thickness (longer for thicker gels); once the bromophenol blue in the dye front has turned yellow, you can proceed with the next step.
2. Remove **Fixing Solution** from the container.
3. Briefly rinse gel with a large amount of Milli-Q water, and remove liquid.
4. Add sufficient **Staining Solution** (just sufficient to completely cover the gel), close container, and rock on platform O/N.
5. Remove **Staining Solution** from container.
6. Wash (destain) gel with multiple changes of Milli-Q water till background staining has been sufficiently reduced.
   Destaining goes faster if you use multiple, short changes of water.
7. If not used, store gel in Milli-Q water in a closed container (labeled with name and date). For storage > 1 week, use **Gel Storage Solution**, and seal in plastic.

Fixing Solution:

50% EtOH	500 ml ethanol
3% H_3PO_4	35.3 ml 85% phosphoric acid
	double-distilled water (Milli-Q) to 1 liter

Staining Solution:

	in this order:
1 g/l Coomassie Brilliant Blue G-250	1 g Coomassie Brilliant-Blue G-250
34% MeOH	340 ml methanol
3% H_3PO_4	25.3 ml 85% phosphoric acid
150 g/l $(NH_4)_2SO_4$	150 g ammonium sulfate
	double-distilled water (Milli-Q) to 1 liter
	Add a sttirring bar, stir O/N, filter solution into clean bottle

Gel Storage Solution:

0.1% HAc	1 ml acetic acid
	double-distilled water (Milli-Q) to 1 liter