

## OHSU PROTEOMICS SHARED RESOURCE GEL PROTOCOL

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### Silver stain

**Uses:** 1D and 2D gels of any size and format, essentially non-quantitative

**Sensitivity:** 5-10 ng

**Notes:** Silver stain is the most sensitive stain currently available. However, the dynamic range and ability to complete downstream analyses are substantial liabilities. Successful protein ID from silver stained gels is possible but has a high failure rate (~50%). A mass spec compatible staining protocol is provided. Use of this stain in any experiment where robust protein ID is desired is absolutely not recommended.

### Protocol:

- 1) The gel is fixed 2 x 30 minutes in a solution containing 5% acetic acid (v/v) and 30% methanol (v/v) in MilliQ water.
- 2) Rinse the gel in MilliQ water for 3 x 10min .
- 3) Gel is then soaked for 1 min in a sensitivity-enhancing solution containing 2 mL of freshly prepared 10% sodium thiosulfate per liter of MilliQ water.
- 4) The gels is rinsed in MilliQ water for 2 x 1 min
- 5) The gel is impregnated for 30 minutes (but no longer than 1 hour) with silver solution containing 0.7 mL of 37% formaldehyde and 12.5 mL of 1 M silver nitrate per liter of MilliQ water. Impregnation should be carried out in an opaque container if possible.
- 6) Gel is then rinsed in milliQ water for 5-15 seconds. This step must be carefully controlled if gel-to-gel reproducibility is required.
- 7) The gel is developed in a solution containing 30g anhydrous potassium carbonate, 0.25 mL of 37% formaldehyde and 1.25 mL of 10% sodium thiosulfate per liter of MilliQ water. Stop development when background begins to appear.
- 8) Development is stopped by placing the gel in a stop solution containing 5% acetic acid for 30-60 min.
- 9) The gel is rinsed with several changes of milliQ water prior to scanning.