

Processing of Silver Stain Gels

It is important to not overdevelop the gel. A staining time of 4-8 minutes is optimal for mass spectrometry applications. DO NOT use the destain procedure in the kit. This does not sufficiently remove all of the silver stain and the gel pieces must be further processed to completely destain. Every washing step will result in some loss of protein so it is important to minimize processing steps. The gel pieces can be destained using the following protocol. You may do this step yourself or submit the gel pieces in 1% acetic acid and we can destain them for you.

Destaining of Silver gels

* Silver stain gel pieces should be stored in 1% acetic acid at 4°C

1. Rinse gel pieces thoroughly with water to remove all the residual acetic acid.
2. Make fresh aqueous solutions of potassium ferricyanide (10mg/ml) and sodium thiosulfate (16mg/ml).
3. Mix the two solutions in a 1:1 ration and immediately add enough to cover the gel pieces. Shake for 10 min at room temp.
4. Discard the supernatant and repeat the destaining until all of the silver-brown color is gone.
5. Once the silver-brown color is removed, wash the gel pieces in 100 mM NH_4HCO_3 for 20 min (sonicating can speed this up). Repeat this wash until the gel pieces are clear.