

The Insite System ~ Order#EC-759

Insite is a unique run-time protein visualization system incorporating a dye/buffer combination which stains proteins during the gel run. The Insite System has been optimized for staining standard Laemmli minigels. Insite will detect as little as 10ng of protein per band.

****Note: Insite Detection Reagent substitutes for standard Tris-Glycine-SDS cathode tank buffer. Do not use with gels requiring other buffers.**

Procedures for Using Insite

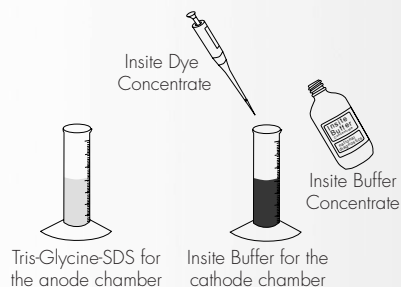
- 1) Assemble gel apparatus.
- 2) Prepare 150 ml of Insite Detection Reagent by diluting 15 ml of 10X Insite Buffer Concentrate to 149 ml with deionized water and adding 1 ml of Insite Dye Concentrate. This solution has a useful life of about 3 hours.
- 3) Fill the anode buffer chamber with 1X Tris Glycine SDS (EC-870) (0.192M Glycine, 0.025M Tris, and 0.1% SDS). Fill the cathode buffer chamber with the Insite Detection Reagent.
- 4) Load the samples.
- 5) Run the gel under standard voltage and temperature conditions. Typical running conditions are 175 volts for 1 hour.
- 6) Remove the gel from the apparatus. [The gel may be observed immediately using a UV transilluminator (see below) for the detection of bands containing more than 300ng of protein.]
- 7) Destain the gel in 50 ml deionized water for 15 minutes. Repeat the wash in fresh deionized water for another 15 minutes.
Alternative Destain: Destain the gel in 50 ml of 0.1M potassium chloride for 30 minutes. Using potassium chloride gives a lower, more uniform background, preserves staining for a longer period, and gives sharper bands.
- 8) Observe the gel on a transilluminator under ultraviolet illumination (302nm). Protein bands fluoresce yellow-orange. If excessive background is observed, wash the gel in an additional deionized water bath for 5-10 minutes.
- 9) Bands may be photographed using the #8 yellow filter used for coomassie stains or the #15 orange filter used for ethidium bromide. Note that background will increase as the gel is allowed to stand and the surface dehydrates. This background is easily removed by a brief wash in deionized water.

Preservation of Stained Gels

- Short Term: The staining of the protein bands may be preserved in deionized water at room temperature for 1-3 hours.
- Extended Preservation: The staining of the protein bands may be preserved in 0.1M potassium chloride destaining solution at room temperature for 12-16 hours with minimal loss in sensitivity.
- Long Term: To preserve the gel staining for a longer period of time transfer the gel to 0.5M potassium chloride after the first 30 minutes of destaining.

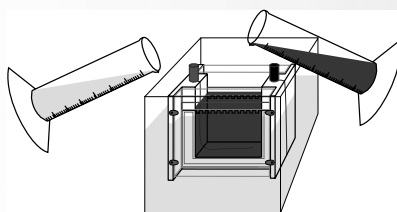
1

Prepare Buffers



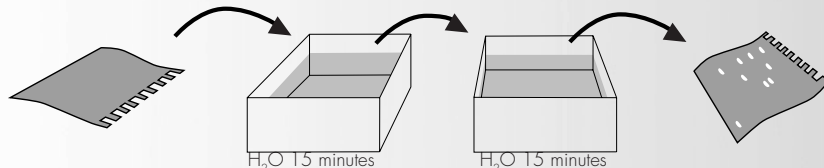
2

Fill Buffer Chambers and Run Gel



3

Destain in Water

Visualize Under UV
Transillumination

Crush and Soak Method for Recovering Proteins from Insite Stained Gels

- 1) Macerate or cut gel slice into pieces less than 1mm in any dimension, in a microcentrifuge tube. The smaller the gel fragments, the faster and more complete the elution will be.
- 2) Add 50 microliters of desired buffer to the tube. Adjust the volume of buffer as needed, so that the gel slice constitutes no more than 25% of the total volume in the tube. The protein will equilibrate between the gel slice and the buffer, so the percentage of total volume made up of added buffer is the maximum possible percentage recovery.
- 3) Soak at 4° C. Recovery increases with soaking time: For 50% recovery, soak for 2-3 hours. For >80% recovery, soak 8 hours or overnight.
- 4) Gel fragments can be removed by filtration or centrifugation, or the supernatant can be pipeted off of the fragments.

****Note: Any dye which co-elutes with the protein may be removed by adding SDS to 0.1%, followed by ultra filtration to remove the free SDS and dye.**

MALDI-MS Analysis of Samples Recovered from Insite Stained Gels

- 1) If necessary, concentrate small amounts of eluted protein until the protein concentration is at least 1-5 picoMole/microliter.
- 2) For the most consistent results use the "crushed crystal" technique of sample spotting:
 - A. Make up a matrix solution of 50% Acetonitrile, 0.1% TrifluoroAcetic acid, saturated with Siniapinic Acid (~40 mg/ml)
 - B. Spot 1 microliter of this solution on the MALDI sample plate, and allow to dry. Crush the resulting crystals by covering them with a glass microscope slide, and applying gentle pressure with the eraser end of a pencil. Remove the slide and gently brush off any loose crystals with a kimwipe, or blow them off with a stream of dry compressed air.
 - C. Mix one part protein sample with 4 parts of the above matrix solution, and spot 1 microliter of this mixture onto the crushed crystals. Allow the sample to dry, and proceed with MALDI-MS analysis.