

Invitrogen SilverQuest Protocol

Visualize down to 3 ng of protein.

Protocol listed below is for one mini-gel, 1.0 mm thick, for large gels double all solution volumes while keeping the same incubation time. **Please do not use the “fast” protocol listed in the SilverQuest manual or the destainer.** Development times between 5 and 6 minutes yield the best mass spectrometry results. Longer development time will result in “burning” of the protein bands, as seen here ([over developed gel](#)). “Burning” decrease peptide recovery which decreases the opportunity for positive protein identification.

| Step | Reagent | Basic Protocol |
|-------------|---|---|
| Fix | Ethanol 40 ml Acetic Acid 10 ml Water to 100 ml | <input type="checkbox"/> 100 ml, 20 min |
| Wash | Ethanol 30 ml Water to 100 ml | <input type="checkbox"/> 100 ml, 10 min |
| Sensitize | Ethanol 30 ml Sensitizer 10 ml Water to 100 ml | <input type="checkbox"/> 100 ml, 10 min |
| First Wash | Ethanol 30 ml Water to 100 ml | <input type="checkbox"/> 100 ml, 10 min |
| Second Wash | Water 100 ml | <input type="checkbox"/> 100 ml, 10 min |
| Stain | Stainer 1 ml Water to 100 ml | <input type="checkbox"/> 100 ml, 15 min |
| Wash | Water 100 ml | <input type="checkbox"/> 100 ml, 1 min |
| Develop | Developer 10 ml Developer enhancer 1 drop Water to 100 ml | <input type="checkbox"/> 100 ml, 4-8 min |
| Stop | Stopper 10 ml Add directly to Developing solution | <input type="checkbox"/> 10 ml, 10 min |
| Wash | Water 100 ml | <input type="checkbox"/> 100 ml, 10 min |