

## Silver Stain Protocol

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last updated: December 10, 2001

**Date:**

**Gel ID:**

**Fix** 3 @ 5 min, 2 @ 45 min, or overnight  
30% EtOH / 10% HOAc (300ml/L & 100ml/L) 1 \_\_\_\_\_ 2 \_\_\_\_\_

**Wash** 3@ 15 min, or 3@ 5 min  
30% EtOH ( 300ml/ L) 1 \_\_\_\_\_ 2 \_\_\_\_\_ 3 \_\_\_\_\_

**Rehydrate** 20 min dd Water \_\_\_\_\_

**Decrease Background** 2 minutes (for 1.5 mm gels: 4 min)  
(*FRESH for 1week*) 2.5 mM NaThiosulfate (0.62 g/L) 0.31 g/500ml  
Time: \_\_\_\_\_

**Wash** 2 @ 5 min dd Water  
1 \_\_\_\_\_ 2 \_\_\_\_\_

**Expose to Silver** 30 minutes 0.2% AgNO<sub>3</sub> 0.2 g/100 ml  
(*Fresh*) plus 0.8 ml 37% Formaldehyde per liter 80 ul /100ml  
Time: \_\_\_\_\_  
**\*\*\* Dispose of in the silver waste container \*\*\***

Wash 1 min dd Water (or longer)

**Revelation** 2-10 min \*\*\* watch closely to get dark enough\*\*\*  
3% Na<sub>2</sub>CO<sub>3</sub> 3g/100 ml  
(*Fresh each time*) plus 0.5 ml formaldehyde 37% 50ul /100ml  
(*Fast shaking*) plus 20uM NaThiosulfate (8ml of 2.5 mM) 800ul/100ml  
Time: \_\_\_\_\_

**Stop** 10 ml Acetic Acid , see lots of bubbles  
**\*\*\*\* Try to anticipate how dark you want it and stop just before this point \*\*\*\***

**Wash** dd Water