Pierce Silver Stain Kit

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Abstract

The Thermo Scientific Pierce Silver Stain Kit is a rapid and ultrasensitive silver stain system for protein detection in polyacrylamide gels. The stain performs consistently and reliably for both first-time and experienced users for high, low or gradient percentage gels in single-dimension or 2-D format. The Pierce Silver Stain Kit provides consistency, high sensitivity, low background and a flexible protocol. Careful manufacturing procedures and thorough quality testing ensure that the kit yields excellent results with every use, independent of the polyacrylamide gel type or buffer system being used. Most proteins are easily detectable at low nanogram or subnanogram amounts, and background levels are very low (see Additional Information section at the end of these instructions). The protocol clearly indicates how both fixing and staining steps can be extended overnight without compromising performance, a feature that enhances convenience for ordinary workday schedules. Likewise, the default 30- minute staining step can be decreased to 5 minutes, enabling the staining procedure to be completed in less than 20 minutes after gel fixation.

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Materials

- Pierce™ Silver Stain Kit 24612 by Contributed by users

Protocol

Step 1.
Wash gel in ultrapure water for 5 minutes. Replace the water and wash for another 5 minutes.

Step 2.
Fix gel in 30% ethanol:10% acetic acid solution (i.e., 6:3:1 water:ethanol:acetic acid) for 15 minutes. Replace the solution and fix for another 15 minutes.

Note: Gel may be kept in fixing solution overnight without affecting stain performance

Step 3.
Wash gel in 10% ethanol solution for 5 minutes. Replace solution and wash for another 5 minutes.

Step 4.
Wash gel in ultrapure water for 5 minutes. Replace water and wash for another 5 minutes.

Step 5.
Prepare Sensitizer Working Solution by mixing 1 part Silver Stain Sensitizer with 500 parts ultrapure water (e.g., mix 50µL Sensitizer with 25mL water)

Step 6.
Incubate gel in Sensitizer Working Solution for exactly 1 minute, then wash with two changes of ultrapure water for 1 minute each.

Step 7.
Prepare Stain Working Solution by mixing 1 part Silver Stain Enhancer with 50 parts Silver Stain (e.g., 0.5mL of Enhancer with 25mL Stain).

Step 8.
Incubate gel in Stain Working Solution for 30 minutes.

Note: Gel may be incubated in Stain Working Solution for as short as 5 minutes or as long as overnight without affecting stain performance.

Step 9.
Prepare Developer Working Solution by mixing 1 part Silver Stain Enhancer with 50 parts Silver Stain Developer (e.g., mix 0.5mL of Enhancer with 25mL Developer)

Step 10.
Prepare 5% acetic acid solution as a Stop Solution.

Step 11.
Quickly wash gel with two changes of ultrapure water for 20 seconds each.

Step 12.

Immediately add Developer Working Solution and incubate until protein bands appear (2-3 minutes).

Note: Protein bands will begin to appear within 30 seconds and then continue to develop. Between 2 and 3 minutes, protein detection vs. background is optimal. After 3 minutes, lane background signal may increase to undesirable levels.

Step 13.
When the desired band intensity is reached, replace Developer Working Solution with prepared Stop Solution (5% acetic acid). Wash gel briefly, then replace Stop Solution and incubate for 10 minutes.