SOP: SP013.1

Coomassie Staining Protocol

Materials and Reagents:

1. Coomassie Brilliant Blue R-250 Staining solution (see SOP: R004)
2. First Fix (see SOP: R002)
3. Coomassie Destaining Solution (see SOP: R005)
4. Pyrex dish
5. Kimwipes (optional)

Protocol:

1._____ Follow SOP SP007 for running SDS-PAGE.

2._____ Wear gloves, as the stain will stain your skin blue. Pour enough stain into a Pyrex dish to completely cover the gel. (It's best to have at least ¼ inch of stain over gel). Remove polyacrylamide gel containing resolved proteins from the electrophoresis plate and place in Coomassie stain.

3._____ Allow gels to rock in Coomassie stain for at least one hour. Some proteins do not stain well with Coomassie. Gels that are > 1 mm thick require 1-3 hours of staining time.

4._____ After staining gel, pour off stain (stain can be reused once). Add enough first fix to cover gel and rock 15-30 minutes.

5._____ Pour off first fix and add approximately 5 to 10 gel volumes of Coomassie destain solution (or 50% Methanol for LC-MS samples). Let rock until background is clear and only protein bands/spots and molecular weight markers are visible. Destaining will take four to eight hours. Adding Kimwipes and replacing them as they stain blue will accelerate the destaining process by soaking up stain. Gels > 1 mm thick require 30 to 60 minutes of destaining in first fix. It is possible to overly destain gels. If this occurs, simply pour out destain solution, put gel back into stain, and start over.

References:

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227: 680-685.

Coligan, J. E., et al. Current Protocols in Protein Science. Volume 2. 10.5.1