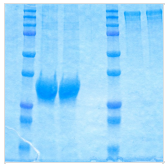

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Colloidal Coomassie Blue Stain: Recipe and Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bmmtk46n

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ABSTRACT

I learned this Colloidal Coomassie Blue staining recipe and protocol when I was a postdoc at Harvard Medical School.. Rumor has it, a postdoc reverse engineered a “safe stain” from a leading commercial supplier. It’s a really great recipe: much more sensitive, faster, and easier to use than traditional methanol based coomassie staining protocols you see in Cold Spring Harbor books, like by Maniatis etc.. Saves a ton of money compared to buying this stuff from commercial vendors. It is downstream compatible with mass spec and most other things you'd want to do with gel slices.

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IMAGE ATTRIBUTION

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GUIDELINES

(1) Be sure you buy Citric Acid Monohydrate. You cannot use salts of Citric Acid.

(2) Be sure you filter (e.g. through a very fine 0.45 um or similar pore size) the 0.5 g/250mL ethanol solution before adding it to the 0.1M citric acid solution, otherwise your final product will cause blue specks to appear on your gels.

(3) When using the microwave steps to do a rapid stain / destain, your goal is to warm up the staining solution + gel but not to cause boiling. So a little less is better than a little more. Don't overdo it.

MATERIALS TEXT

Coomassie Brilliant Blue G
Ethanol (95%-100% pure)
Citric Acid Monohydrate (do NOT use sodium or other salts of citric acid)

Distilled Water

DISCLAIMER:

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>
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BEFORE STARTING

Make sure you have:

1. Coomassie Brilliant Blue G (Coomassie Brilliant Blue R isomer gives a different, more purple/ violet color
2. Stir bars
3. 200 proof ethanol (95% ethanol probably OK as a substitute)
4. Citric Acid Monohydrate (do not use sodium or other salts of citric acid).
5. Graduated cylinders
6. 0.45 um bottle top filters or similar

1 Dissolve 0.5 grams Coomassie Brilliant Blue G powder in 250 mL ethanol (200 proof), mix very well on stir bar until it seems fully or very nearly fully dissolved.

2 Filter the Coomassie Blue G in ethanol solution through 0.2 or 0.45 um membrane, like a bottle top vaccum filter, to produce a solution that is free of any insoluble Brilliant Blue particles (Do not skip this step!)

3 In a large graduated cylinder or other suitible container, 4.75 L of distilled (or pure) water, dissolve 105.1 grams of citric acid monohydrate (sufficient material to produce a 0.1 M citric acid monohydrate solution when brought to 5 L.

4 Dump the filtered 250 mL of ethanol containing 0.5 grams of dissolved Brilliant Blue G into the 4.75 L citric acid monohydrate solution, bringing it to 5L of 0.1 M citric acid monohydrate and 0.1% Brilliant Blue G. Mix well.

5 To stain an SDS-PAGE protein gel using this staining reagent, gently transfer an SDS-PAGE gel into ~50-100 mL of distilled or deionized water and microwave for approximately 30-45 seconds, until the water is very warm but not boiling. Then carefully dump off the water and replace with 50-70 mL of colloidal coomassie blue staining solution (the product of step 4). You want just enough to fully cover the gel. Microwave the gel + stain solution again for 30-45 seconds or until very warm. Let the gel rock on a rocking platform for 3-10 minutes while checking occassionally how the bands are developing. Once fully stained (takes usually no longer than 15 min) you can dump off the staining solution and replace with 100-150 mL of water. Microwave the gel in water for 45 seconds to heat it, and rock on a rocking platform with a tiny crumpled piece of kim wipe or paper napkin to accelerate destaining process. You can change out the water and keep replacing the kimwipe until the destaining is complete enough. Or just leave it out on your bench overnight in water.. the will destain nicely.

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