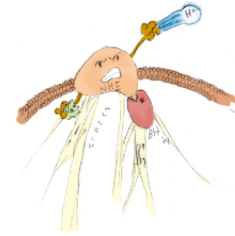


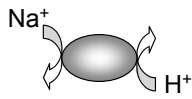
General Cell Ab Labeling Protocol



Introduction: General protocol when staining cultured cells.

General Antibody Labeling Protocol.

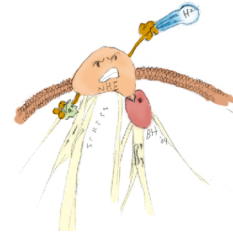
1. Rinse cells in buffer (HBSS - Hanks Buffered Saline Solution, or PBS - Phosphate Buffered Saline, or Tyrodes + HEPES) at 37°C to remove all culture media. Keep buffer warm to prevent heat shocking the cells which may make them detach.
2. Fix samples in warm (37°C) 3.7% formaldehyde, diluted in buffer; incubate 10-15 minutes at room temperature. The quality of the aldehyde is very important for good results. We recommend Polysciences Cat# 18814, Formaldehyde 16% Ultrapure (Methanol Free). Slightly longer fixations (20-30 mins) maybe acceptable if it does not disrupt the antigenicity of target.
3. Rinse samples 3-4 times, for one minute each, in buffer. For cells grown on coverslips you can rinse them through several beakers of buffer for 15-20 seconds each.
4. Permeabilize cells in 0.2% Triton X-100, diluted in buffer, for 5 minutes or 0.1% Triton X-100 for 15 minutes.
5. Rinse samples 3-4 times in buffer.
6. For antibody labeling block cells with suitable blocking agent. For general purposes we recommend BSA (Fraction V, lipid free) between 1-3% in buffer at room temp or 37°C for 30-60 minutes. More complex blocking agents can be used such as 5-10% filtered goat serum or Fetal Calf serum if desired. If using whole serum make sure you centrifuge and syringe filter the serum to remove particulate material.
7. Prepare antibody dilutions in the same blocking solution as above. Optimal concentration of primary will have to be determined experimentally but as a general rule 2-5 µg/mL will work for many monoclonal antibodies. Incubate 60 minutes at room temp or 37°C in humidified chamber.
8. Rinse samples 3-4 times with buffer. If desired buffer + 0.05% Tween-20 can be used.
9. Prepare secondary antibodies in same blocking solution as above. Optimal concentration should be determined experimentally but as a general guide:
 - General fluorophore labeled secondaries between 5-10 µg/mL.
 - Alexa Fluor labeled secondaries between 2-5 µg/mL.
 - Enzyme conjugated secondaries between 2-5 µg/mL.Incubate 60 minutes at room temp or 37°C in humidified chamber.
10. Rinse samples 3-4 times with buffer. If desired buffer + 0.05% Tween-20 can be used.



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General Cell Ab Labeling Protocol



11. If no further staining is required proceed with mounting the sample. For quick observations the specimen can be mounted in buffer and the coverslip secured using hot wax or nail polish. Generally it is better to use an antifade mounting agent such as SlowFade or SlowFade Light. These are water miscible glycerol based solutions. Simply apply a drop to the slide and place the coverslip specimen side down onto the drop. The coverslip can be attached using hot wax or nail polish. Sealing the coverslip is recommended if you intend to use oil immersion objectives.
12. For more permanent mounting one can use an agent such as CytoSeal (requires ethanol dehydration) or Prolong.

Are formaldehyde and formalin the same substance?

Formaldehyde, in its basic form, is a gas. Most people think of formaldehyde as a liquid. The liquid is actually a mixture of formaldehyde gas and water. The most common concentration used is a 37% solution. That solution contains 37 grams of formaldehyde gas to 100 ml of solution. Formaldehyde solution will polymerize. To prevent polymerization of formaldehyde solution, about 10 - 15% of methyl alcohol is added. It is the addition of methyl alcohol that causes the substance to be called formalin as opposed to formaldehyde.

We have occasionally encountered some confusion about the difference between formaldehyde and formalin. This is an understandable problem, since the terms are sometimes used interchangeably. It is incorrect to use the two words this way. The concentrations of chemical fixative that the two names represent are quite different. A fixative labeled as 10% buffered formalin is actually only a 4% solution of formaldehyde. This is because 10% buffered formalin is an example of old-time histologist's jargon describing a 10% solution made from a stock bottle of 37-40% formaldehyde (or more precisely: a 3.7-4% solution of formaldehyde).