Blocking in immunofluorescence

What blocking solution should I use for Nano-Secondaries?

Content

1. Introduction

Blocking is an essential step during the preparation of a sample for immunofluorescence detection; blocking improves the sensitivity by reducing nonspecific background and therefore increases image quality. Insufficient blocking will result in higher background noise and over-blocking can even mask the specific signal.

In order to prevent non-specific interactions of the primary and secondary antibody reactive sites of the sample are covered/blocked. In theory, any protein that does not bind to the target antigen can be used as blocking reagent. In fact, sera blocking solutions and protein blocking buffers are most frequently used.

2. Common blocking buffers

2.1. Blocking with serum

Serum contains multiple of proteins, which block non-specific epitopes in the sample. In addition, sera carry antibodies that will bind to Fc receptors of the sample tissue and prevent primary and secondary antibodies from doing the same, which increases background.

2.2. Blocking with proteins

Protein blocking solutions consist of highly concentrated proteins like BSA, casein or skimmed milk / nonfat dry milk in buffer. The proteins outcompete for the non-specific binding to the sample with the primary and secondary antibodies.

3. Blocking recommendation for immunostaining with Nano-Secondaries

Nano-Secondaries™ are secondary single domain antibodies derived from alpacas. Since Nano-Secondaries lack the Fc part of a conventional antibody, blocking with alpaca (and also llama) serum to minimize binding to Fc receptors is not required. Instead, standard protein blocking solutions e.g. 4% BSA or skimmed milk in PBS are sufficient when using Nano-Secondaries for immunostaining.

4. Blocking protocols

Although laboratory praxis may apply established blocking protocols, blocking should be optimized for each experiment. Empirical testing is critical to obtain best results for a given combination of specific antibodies and sample composition.

4.1. General blocking protocol

1. Fixation and permeabilization of the sample
2. Blocking (immediately prior to incubation with primary antibody):
   - Serum at 1-5% in buffer OR proteins at 1-5% in buffer
   - 20-60 minutes up to overnight
   - at +4°C or ambient temperature
3. Wash
4. Incubation with primary antibody. If the primary antibody is diluted in blocking solution, washing step (3.) can be omitted.
5. Wash
6. Incubation with secondary antibody
7. Wash
8. Counterstain with DAPI, mount, imaging

4.2. Blocking protocol for sequential staining with primary antibodies and Nano-Secondaries

1. Fixation and permeabilization of the sample
2. Blocking (immediately prior to incubation with primary antibody and Nano-Secondary):
   - proteins at 1-5% in buffer (e.g. 4% BSA in PBS)
   - 20-60 minutes up to overnight
   - at +4°C or ambient temperature
3. Dilution of primary antibody in blocking solution
4. Incubation with primary antibody
5. Wash
6. Dilution of Nano-Secondary in blocking solution
7. Incubation with Nano-Secondary
8. Wash
9. Counterstain with DAPI, mount, imaging

4.3. Blocking protocol for simultaneous one-step staining with primary antibodies and Nano-Secondaries

1. Fixation and permeabilization of the sample
2. Blocking (immediately prior to incubation with primary antibody and Nano-Secondary):
   - proteins at 1-5% in buffer (e.g. 4% BSA in PBS)
   - 20-60 minutes up to overnight
   - at +4°C or ambient temperature
3. Dilution of primary antibody together with Nano-Secondary in blocking solution
4. Simultaneous incubation with primary antibody and Nano-Secondary
5. Wash
6. Counterstain with DAPI, mount, imaging