abcam

Mouse on Mouse (MOM) staining procedure

Tips for eliminating background when staining mouse tissue with a mouse monoclonal.

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Overview

Staining of mouse tissue using mouse antibody is a complicated process as high levels of background are often observed. It is notoriously difficult to eliminate this background.

Much of the background is caused by secondary antibody binding to endogenous mouse IgG in the tissue being stained, and to Fc receptors on B cells, plasma cells and macrophages.

Abcam offers a robust kit to allow the use of mouse antibodies on mouse tissue. Mouse on Mouse Polymer IHC Kit (ab269452) is a polymer based system that provides increased sensitivity, detection simplicity, and saves time.

However, there a few tips to try and reduce this background should mouse on mouse staining be necessary.

Blocking of endogenous IgG

1. Prepare tissue sections as usual.
2. At the usual blocking step, block with serum (from same species as the secondary antibody) for 30 min at room temperature.
3. Wash 3 X 2 min with PBS Tween 20. Incubate sections with an unconjugated affinity purified F(ab) fragment anti-mouse IgG (H+L) for 1 hr at room temperature, or overnight at 4°C. Try this blocking antibody at 0.1 mg/ml although the optimal concentration will need to be assessed by the end user.
4. Proceed with antibody staining.

Blocking endogenous Fc receptors

Fc receptors are present on several cell types such as macrophage, and monocytes and can bind to the antibody and give additional background staining. We recommend using F(ab) monomeric secondary antibodies to help reduce background.

Other tips that can be used to decrease general background:

1. Incubate sections with 1% Triton (in PBS) at room temperature for 30 min to 'clean' the tissue.
2. Use TBS-Tween 20 as a washing buffer. This often gives less background than using PBS-Tween 20.