abcam

Whole mount troubleshooting tips for your IHC and ICC experiments

Overview

Very similar difficulties to immunocytochemistry (ICC) and immunhistochemistry (IHC) can occur when staining whole mount tissue. We can recommend reviewing the ICC and IHC troubleshooting tips provided on the following page:

http://www.abcam.com/ps/pdf/protocols/abcam%20troubleshooting%20tips%20-%20IHC.pdf

The following tips are more specific to whole mount staining. Most of these relate to the fact that incubation times for all reagents, and wash steps, need to be much longer that in ICC or IHC to allow penetration through the sample, which will be much larger than a tissue section.

High background

Fixative used is not suitable for the antibody

Most researchers use PFA for fixation. As antigen retrieval methods are not recommended for whole mount (it can destroy the tissue), ensure the concentration of this is no more than 4%. This should cause fewer difficulties with protein crosslinking. Some antibodies will still be sensitive to the small amount of protein crosslinking at this lower percentage PFA, and PFA fixation will not be suitable for some antibodies.

The usual alternative to PFA is methanol fixation. However, we would recommend checking the antibody datasheet to obtain information on fixation agents used successfully in whole mount sections with the antibody you are using. If this information is not available, fixatives used successfully in cryosections are usually successful in whole mount.

Fixation time may also require optimization.

Antibody left on for too long

The recommendation when optimizing antibody concentration in whole mount is to start with 3 or 4 day incubation. If this is not successful, work backwards to 1 day (antibody will need to be on the sample for at least 24 hours to ensure full penetration through the sample).

Microbial contamination

As the incubations in whole mount staining are very long, microbial contamination can become a problem. This can lead to non specific background staining. Use clean glassware (preferably sterile) and fresh reagents. 0.2% sodium azide can be added to antibody buffers and blocking buffers wherever possible. Please note this should not be added to peroxidase conjugated secondary antibodies as it can inhibit the enzyme acitivity. Follow washing guidelines carefully before adding peroxidase conjugated secondary antibody to ensure any sodium azide is washed away.

Wash steps not sufficient

Follow the wash step guidelines provided in the protocols. Wash steps will need to be long enough to permeate and wash through the whole sample. Triton rather than Tween is used as this is a stronger detergent which will permeate more easily.

No signal

Antibody not left on for long enough

Antibody incubations should be much longer than when staining sections on slides to ensure adequate permeation to the centre of the sample. The recommendation when optimizing antibody concentration in whole mount is to start with 3 or 4 day incubation. If this is not successful, work backwards to 1 day (antibody will need to be on the sample for at least 24 hours to ensure full penetration through the sample).

Incorrect incubation times

Incubations for fixative, blocking buffer, antibody, wash buffer, permeabilization and substrate color development will need to be much longer to allow for permeabilization right into the centre of the sample.

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Fixation time may also require optimization.

Incorrect detergent used in buffers

In order to allow full permeabilization of reagents and antibody through the whole sample, Triton rather than Tween is normally used. This is a stronger detergent which will permeate more easily. It is also used at a relatively high concentration of 0.5 to 1%.

Zebrafish – egg membrane not permeabilized

Whole mount staining of Zebrafish embryos requires extra steps to fix and permeabilize to ensure the egg membrane is permeabilised. Fix for 1 hour, wash in PBS 1% triton then permeabilize the egg membrane in in ice cold acetone / PBS for 8 minutes only. We recommend following the zebra fish whole mount staining procedure provided.

Mouse and chick – extracellular membrane not removed

Remove as much embryonic membrane and excess tissue as possible as this can prevent the antibody perfusing into the embryo.

Patchy staining

Incorrect incubation times

Incubations for fixative, blocking buffer, antibody, wash buffer, permeabilization and substrate color development will need to be much longer to allow for permeabilization right into the centre of the sample. If any of these reagents have not penetrated the whole sample, there may be areas of the tissue not fully washed, fully fixed, or with full access to the antibody. This will lead to patchy areas where staining is not sufficient.

Air bubbles in the tissue / Inadequate mixing of reagent and sample

Ensure the sample is placed on a gentle rotating or rocking devise whilst incubating to prevent formation of air bubbles and to ensure access of reagent to all the tissue. If any of the reagents have not penetrated the whole sample, there may be areas of the tissue not fully washed, fully fixed, or with full access to the antibody. This will lead to patchy areas where staining is not sufficient, or trapped airbubbles in which tissue will not be stained.

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Sample is too large

An embryo will grow to a stage where they are too large for whole mount staining with good results, and other types of sample will need to be kept within an optimized size range. Reagents will not be able to fully penetrate the tissue if it is too large, and the staining through the sample will be patchy. It is sometimes possible to dissect the sample into sections which are more manageable.

Morphology of the tissue is not good

Inadequate fixation or overfixation

Ensure the sample has been fixed for long enough to allow penetration of fixative through the sample. The timing of fixation may require some optimization. Fix at 4oC. We suggest optimizing between 2 hours and overnight.

Sample has been heated or treated for antigen retrieval

Heat treating the whole mount samples for antigen retrieval is not possible, as it destroys the structure of the tissue. Try using another fixative, rather than PFA. The usual alternative to PFA is methanol fixation. However, we would recommend checking the antibody datasheet to obtain information on fixation agents used successfully in whole mount sections with the antibody you are using. If this information is not available, fixatives used successfully in cryosections are usually successful in whole mount.

Sample has been crushed whilst handling

To move the tissue from one vial to another, or to add or remove reagents, use a plastic Pasteur pipette with the tip removed (this prevents any damage to the embryo from the narrow end of the pipette). Avoid use of forceps where possible.