GETTING STARTED GUIDE

READ THIS FIRST BEFORE WORKING WITH Visikol® HISTO™

Our products are designed to make tissue clearing and visualization easy, but this skill is an art that requires a little patience and diligence to learn to use effectively. Please read this guidebook and pay attention to these important considerations and preparation steps before you proceed with staining, clearing, and imaging tissue samples.

Label Optimization → Permeabilitzation → Labeling → Clearing → Imaging

Getting the most out of Visikol HISTO depends on your labeling method.

Fluorescent Protein

Unless combined with immunolabeling, there is no need to perform permeabilitzation or labeling steps.

Proceed directly to clearing and perform dehydration with ethanol at 4°C (see reverse side).

Nuclear & Viability Stains

Apply stain as directed by manufacturer, fix tissue, and proceed directly to dehydration and clearing (see reverse side).

Antibody Labeling

Optimizing antibody dilution is the most important step to getting good results with clearing!

Optimization at a Glance

Try a dilution on 100 µm section → Examine evenness & depth → Scale incubation time with tissue thickness

A few days optimizing staining for your tissue can save weeks of wasted effort.
Please read more about the factors that can affect immunolabeling in the guidebook.

1. Optimizing dilution: Cut three to five 100-200 µm tissue sections to explore dilutions ranging from 1:500 – 1:50. Smaller sections can be stained and cleared in a single workday (see reverse side)
2. Cut perpendicular cross-sections of the tissue slice to examine evenness and depth of penetration of stain.
3. Antibody concentration is a balancing act: too low and there will be low signal to noise, too high and the outer layers will "shadow" the inner layers due to absorbance in the outer layers.
4. Volume of antibody solution should completely cover tissue of interest.


Optimum concentration


Concentration too high


Concentration too low

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Permeabilitzation

		100µm section	Hemi-sphere
2x	PBS	5 min	30 min
1x	50% MeOH	-	1 h
1x	80% MeOH	-	1 h
1x	100% MeOH	15 min	1 h
1x	5% H2O2/20% DMSO/ MeOH at 4°C	30 min	12 h
	Optional: For tissues containing blood or pigment		
2x	20% DMSO/MeOH	15 min	1 h
1x	100% MeOH	15 min	1 h
1x	80% MeOH	-	1 h
1x	50% MeOH	-	1 h
1x	PBS w/ 1% Triton X-100	5 min	30 min

Labeling

		100µm section	Hemi-sphere
1x	Penetration Buffer	15 min	36 h
1x	Blocking Buffer	15 min	36 h
1x	Antibody Buffer	30 min	48 h
	Add nuclear stain in this step if desired		
5x	1X Wash Buffer	5 min	30 min
1x	Antibody Buffer	30 min	48 h
	Secondary antibody staining, if using indirect labeling		
5x	1X Wash Buffer	5 min	30 min
1x	PBS	5 min	30 min

Clearing

		100µm section	Hemi-sphere
1x	50% MeOH	-	1 h
1x	80% MeOH	-	1 h
1x	100% MeOH	30 min	1 h
1x	HISTO-1	30 min	16 h
1x	HISTO-2	30 min	16 h

Included with the Starter Kit

- HISTO-1
- HISTO-2
- Antibody
- Blocking Buffer
- Penetration Buffer
- 10X Wash Buffer
- Permeabilization Buffer

Imaging

1. Use silicon Visikol ClearWell™ of appropriate size for tissue (alternatively, Sticky Tac can be used to create a well on a slide).
2. Mount samples in HISTO-2 and coverslip.
3. While all confocal microscopes are compatible with Visikol HISTO, using an air lens will limit imaging depth to less than 2mm.
4. Water, glycerol, and CLARITY optimized objectives should be used with a double-chambered cuvette; mount samples in HISTO-2 in the inner cuvette, seal, and fill outer cuvette with water/glycerol mixture to match RI of objective.
5. Large whole tissues (e.g. mouse brain) should be imaged using a light sheet micro-scope.