GETTING STARTED GUIDE

READ THIS FIRST BEFORE WORKING WITH Visikol® HISTO-M™

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 labeling, clearing, and imaging tissue samples.

Label Optimization → Permeabilitzation → Labeling → Clearing → Imaging

Getting the most out of Visikol HISTO-M 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).

Immunolabeling

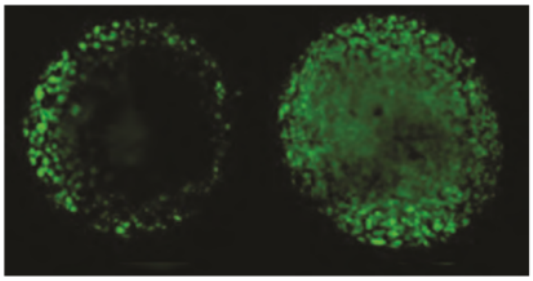
Optimizing antibody dilution is the most important step to getting good results with clearing!

Optimization at a Glance

Try conditions on a single culture → Examine Uniformity & Depth

A few days optimizing staining for your tissue can save weeks of wasted effort

1. Optimizing dilution and time: Use at least six in vitro models to explore dilutions ranging from 1:500 – 1:50 and times from 30 minutes to 12 hours.
2. Image using confocal microscope and look for even staining in Z-sections.
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.



Non-uniform Labeling **Uniform Labeling**

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Permeabilitzation

		3D cell culture
1x	PBS	15 min
1x	MeOH	15 min
1x	20% DMSO/MeOH	15min
1x	MeOH	15 min
1x	PBS w/ 1% Tx100	15 min

Important: Make sure that 3D cultures have settled to the bottom of the well before exchanging solutions.

Labeling

		3D cell culture
1x	Penetration Buffer	15 min
1x	Blocking Buffer	60 min
1x	Antibody Buffer	**30 min***
	Add nuclear stain (optional)	
5x	1X Wash Buffer	15 min
1x	Antibody Buffer	**30 min***
	Add secondary antibody (optional)	
5x	1X Wash Buffer	15 min
1x	PBS	15 min

Note: Antibody incubation time will vary from 30 minutes to 12 hours depending on the model.

Clearing

		3D cell culture
1x	MeOH	15 min
1x	HISTO-M	15 min

Included with the Starter Kit

- Visikol® HISTO-M™
- Antibody Buffer
- Blocking Buffer
- Penetration Buffer
- 10X Wash Buffer

Imaging

1. Once cleared with Visikol HISTO-M, 3D cell culture models should be left in a well plate or transfered to a slide in Visikol HISTO-M for imaging.
2. 3D cell culture models cleared with Visikol HISTO-M can be imaged with confocal or widefield microscopy. While Visikol HISTO-M will allow for a substantial increase in the number of cells characterized with widefield microscopy, optical Z sectioning will not be possible as it is with confocal microscopy.
3. The ideal imaging systems for imaging 3D cell culture models are high content confocal imaging systems such as the Perkin Elmer Phenix Opera/Operetta, Cell Insight CX7 LZR/LED, GE IN CELL 6000/6500, or the Molecular Devices ImageXpress.
4. For optimum image quality, use glass, flat-bottom well plates (e.g. Corning Cat# 4580) in a high content confocal imaging system with laser excitiation.