Mounting Medium Types for Immuno-fluorescence Microscopy

To obtain accurate and reproducible results from immunofluorescence imaging applications, it is essential to maximize the intensity and stability of the fluorescence signal in the experimental sample. Loss of fluorescence through irreversible photobleaching processes can lead to a significant reduction in sensitivity, particularly when target molecules are of low abundance or when excitation light is of high intensity or long duration. To minimize photobleaching of experimental samples, the use of a mounting medium is recommended. The fading characteristics of mounting media for fluorescence microscopy are influenced by adding compounds, such as antioxidants and free-radical scavengers. These compounds interfere with the photochemical reaction in such a way that the excited fluorochrome will not be damaged. One well-known additive that considerably reduces fading by acting as a free-radical scavenger is 1,4–diazabicyclo[2.2.2]octane (DABCO).

A mounting medium should have the following properties:

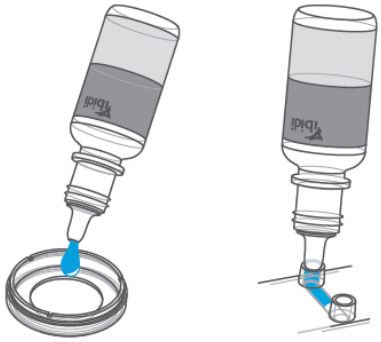
- A refractive index close as possible to 1.5,
- It should be colorless and transparent,
- It should not cause the stain to diffuse or fade (the choice of the mounting medium is dependent on the staining protocol used),
- It should have no adverse effect on cells or tissue components,
- It should protect the sample from physical and chemical activity (e.g. oxidation),
- It should set without crystallizing, cracking or shrinking (for hardening mounting media).

Table 1 Comparison of non-hardening and hardening mounting medium

	Non-hardening mounting medium	***Hardening mounting medium***
Usage	Ready-to-use liquid; Samples can be viewed immediately	Mounting Medium has to set before samples can be viewed
Shrinking	No shrinking	Shrinking possible during setting process, therefore not suitable for closed formats
Storage	Medium-term storage	Long-term storage
Recommended for	µ-Slides and µ-Dishes	Coverslip fixation such as for 3, 8, and 12 Well Chamber, removable

Application of a non-hardening mounting medium

ibidi Mounting Medium is a non-hardening mounting medium. It can be applied to your samples which can be viewed immediately. ibidi Mounting Medium is recommended for all µ-Slides and µ-Dishes.



Easy well filling (e.g., µ-Dish) *Easy channel filling (e.g., µ-Slide VI)*

Application of a hardening mounting medium

The general workflow for the use of a hardening mounting medium is as follows: The mounting medium is applied to your sample and a coverslip is placed on top. The mounting medium has to set before the samples can be viewed. Hardening mounting medium is recommended for the 3, 8, and 12 Well Chamber, removable.