

CelExplorer Labs

Product Information

FocusClearTM

A water-soluble clearing agent making biological tissue transparent

FC-101 *FocusClearTM*

MC-301 *MountClearTM*

IS-502 *Immersion Solution-M*

1. INTRODUCTION

Why use *FocusClearTM* Fluorescence labeling is essential for fluorescence microscopy and confocal microscopic analysis of biological samples. Since many fluorophores are quenched or extracted during routine dehydration procedures, most fluorescence-labeled samples are directly mounted in glycerol-based mounting media for fluorescence and confocal imaging. Under such conditions, a biological structure can be viewed at about 100 μ m beneath the surface using laser source with wavelength at visible spectrum range. To increase depth of view, one can use two-photon confocal system with infrared laser as light source and viewing structures twice deeper than the conventional confocal microscope. *FocusClearTM* is a water-soluble clearing agent for increases the transparency of biological tissues. As a result, image quality of fluorescence or non-fluorescence labeled specimens cleared in the *FocusClearTM* is greatly improved. *FocusClearTM* facilitates light penetration and allows visualization of internal objects up to at least 500 μ m below tissue surface. In contrast, traditional clearing agents such as glycerol-based mounting media allow visualization of only up to 150 μ m inside the specimen. Because of high tissue transparency, *FocusClearTM* also increases the efficacy of laser excitation and optical signal detection of either color or fluorescence. *FocusClearTM* is suitable for microscopic observation of immunofluorescence-labeled single cells and tissues, *in situ* hybridization, tissue or whole-mount immunohistochemistry, and fluorescence protein samples.

Properties: *FocusClearTM* solution is a water-soluble clearing agent. It is not gelling in the bottle and no dehydration of the objects is necessary. Samples can be directly transferred from water, buffer solutions, alcohol, DMSO, DMF, and glycerin into *FocusClearTM* solution. *FocusClearTM* can be used for samples labeled with fluorescence and non-fluorescence dyes including lipophilic dyes, such as DiI, DiD and NBD-ceramide. *FocusClearTM* is non-toxic, ready to use, always liquid, no need to be aliquoted, mixed, centrifuged or kept frozen. It allows easy and universal production of preparations.

MountClearTM is a mountant specially designed for mounting specimens cleared by the *FocusClearTM*. *MountClearTM* does not interfere the clearing effect of *FocusClearTM*. In addition, it has anti-quenching, non-fluorescence and quick clotting characteristics. Using mounting media other than *MountClearTM* may result in cloudiness of the sample.

Immersion Solution-M is an immersion solution with a refraction index matching to those of *MountClearTM*. They are designed to avoid deformation of the observed images during high-resolution microscopic observation using oil or water immersion objective lens.

Effects: Tissues in the *FocusClearTM* become transparent. The resolution and depth of focus greatly increased with sharp outline and high contrast. In order to obtain best results, it is recommended that the specimen cleared in *FocusClearTM* should be mounted in *MountClearTM* and observed with oil or water immersion lens with high numerical aperture and covered with *Immersion Solution-M*. *FocusClearTM*, however, is designed to clear specimens fixed by cross-linking agents such as paraformaldehyde and glutaraldehyde and it is ineffective for heat-denatured or alcohol fixed specimens.

Applicable tissues: Mouse brain, insect brain, human tumors, some plants tissues.

2. STORAGE AND HANDLING

Store at room temperature. Do not freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by incubation in hot-water bath followed by centrifugation before use.

3. WARNING AND PRECAUTIONS

These products are intended for research purposes only. They may contain materials that are toxic to humans and animals, and should not be administered either externally or internally.

4. APPLICATION PROTOCOL

1. Paraformaldehyde and/or glutaraldehyde fixed samples labeled with immunofluorescence, fluorescence probes,

immunohistochemicals, or conventional dyes are thoroughly washed to remove non-specific bindings.

2. Tissue blocks, brain slices, cryosections or single cells ready for microscopic observation can be directly transferred into appropriate amount of *FocusClearTM* solution for clearing. **Note:** For an intact fly brain, 100 μ l *FocusClearTM* solution is suggested. For a slice of mouse brain (200 μ m thick), 1 ml *FocusClearTM* solution is suggested.
3. For an effective clearing, the incubation time (10 min to 4 h) should be adjusted according to the size of the tissue ($10^6 \mu\text{m}^3 \sim 1 \text{mm}^3$). To prevent evaporation during clearing, the incubation chamber should be completely sealed with parafilm membrane. **Note:** Small samples such as fly brains may become completely transparent and difficult to be retrieved under a dissecting microscope. By simply applying a drop of saline solution, your precious samples will become visible again. You can clear the tissue again in a smaller drop of *FocusClearTM* for easy retrieval.
4. The cleared specimens are then mounted in a fresh drop of *FocusClearTM* solution.
5. For the best quality, the cleared specimens should be mounted in the *MountClearTM* solution. Prior to every

use, the *MountClearTM* solution should be completely dissolved again by incubation in the hot-water bath (55 °C) for about 30 min. After brief cooling at room temperature, the *MountClearTM* solution is ready for use. For a longer operation, keeping the *MountClearTM* solution at 37 °C to maintain its fluidity is suggested.

6. Seal the sample completely with fingernail polisher.
7. When using an oil/water immersion lens to observe the sample, *Immersion Solution-M* matching the reflective index of the mounting solution should be used for better resolution.

5. TECHNICAL ASSISTANCE

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6. REFERENCES

1. J. Comp. Neurol. (1999) 413, 593-602.
2. J. Comp. Neurol. (2001) 440, 1-11. (cover picture)
3. Proc. Natl. Acad. Sci. (2002) 99, 37-42.

Product List

Cat. No.	Product name	unit size
FC-101	<i>FocusClearTM</i>	5 ml
MC-301	<i>MountClearTM</i>	5 ml
IS-502	<i>Immersion Solution-M</i>	5 ml

CelExplorer's products are high-quality reagents for laboratory use only. These reagents are not for drug, household or other uses. Most CelExplorer's products and product applications are covered by U.S. and international patents and patents pending.