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Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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CelExplorer Labs

Product Information

FocusClear™

A water-soluble clearing agent making biological tissue transparent

FC-101 *FocusClear™*

MC-301 *MountClear™*

IS-502 *Immersion Solution-M*

1. INTRODUCTION

Why using *FocusClear™* is essential for fluorescence microscopy, confocal microscopic analysis or extracting 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 100μm beneath the surface by 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. *FocusClear™* 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 is greatly improved. *FocusClear™* facilitates light penetration and allows visualization of internal objects up to at least 500μm below tissue surface. In contrast, traditional clearing agents such as glycerol-based mounting media allow visualization of only up to 150μm inside the specimen. Because of high tissue transparency, *FocusClear™* also increases the efficacy of laser excitation and optical signal detection of either color or fluorescence. *FocusClear™* 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: *FocusClear™* 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 *FocusClear™* solution. *FocusClear™* can be used for samples labeled with fluorescence and non-fluorescence dyes including lipophilic dyes, such as DiI, DiD and NBD-ceramide. *FocusClear™* 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.

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

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

Effects: Tissues in the *FocusClear™* 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 *FocusClear™* should be mounted in *MountClear ™* and observed with oil or water immersion lens with high numerical aperture and covered with *Immersion Solution-M*. *FocusClear™*, however, is designed to clear specimens fixed by cross-linking agents such as paraformaldehyde and glutaraldehyde, but 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 4℃. Do not freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by incubating in hot-water bath and centrifugation should be followed 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 should be

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 *FocusClear™* solution for clearing.
   Note: For an intact fly brain, 100µl *FocusClear™* solution is suggested. For a slice of mouse brain (200 µm thick), 1 ml *FocusClear™* 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 μm^3 ~ 1 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 *FocusClear™* for easy retrieval.
4. The cleared specimens are then mounted in a fresh drop of *FocusClear™* solution.
5. For the best quality, the cleared specimens should be mounted in the *MountClear™* solution. Prior to every use, the *MountClear™* solution should be completely dissolved again by incubation in the hot-water bath (55℃) for about 30 min. After brief cooling at room temperature, the *MountClear™* solution is ready for use.
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 ASSITANCE

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6. REFERENCES

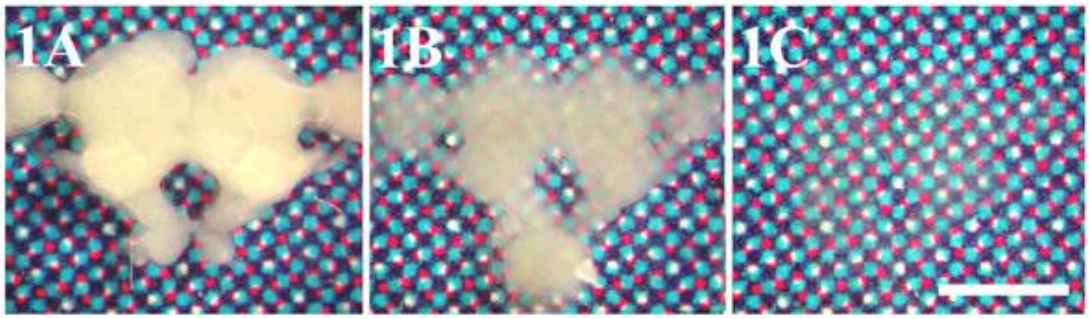
1. J. Comp. Neurol. (1999) 413, 593-602.
2. J. Comp. Neurol. (2001) 440, 1-11. (cover picture)
3. Proc. Natl. Acad. Sci. (2002) 99, 37-42.
4. Gastroenterology. (2009) 137(2), 453-65.

Product List

Cat. No.	Product name	unit size
FC-101	*FocusClear™*	5 ml
MC-301	*MountClear™*	5 ml
IS-502	*Immersion Solution-M*	5 ml

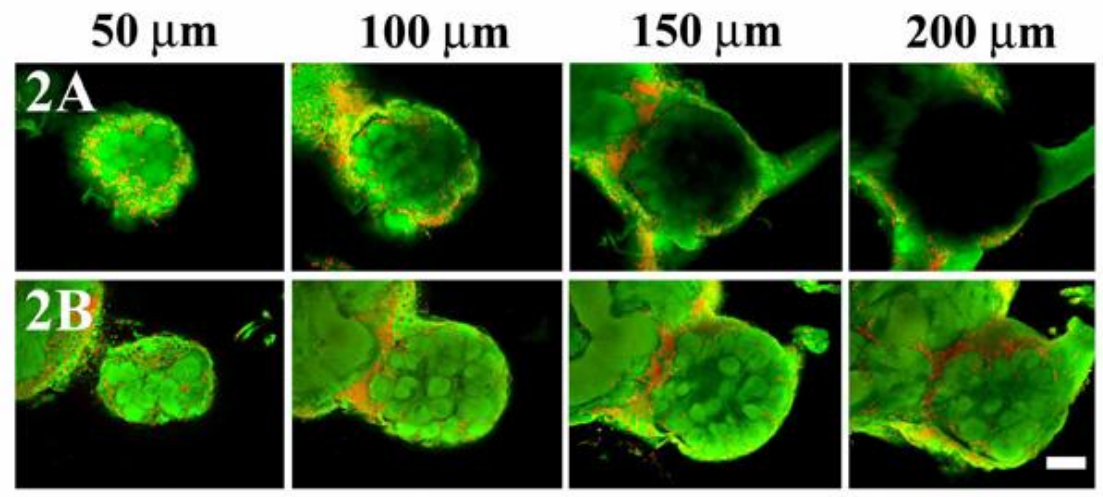
CelExplorer's products are high-quality reagents for laboratory use only. These reagents are not for drug, household or other uses. Most CelExplorer's products and product applications are covered by U.S. and international patents and patents pending.

Demonstration



The FocusClear technique has a dramatic effect on the transparency of soft biological tissues. A brain tissue from cockroaches becomes invisible when merged in the FocusClear fluid.

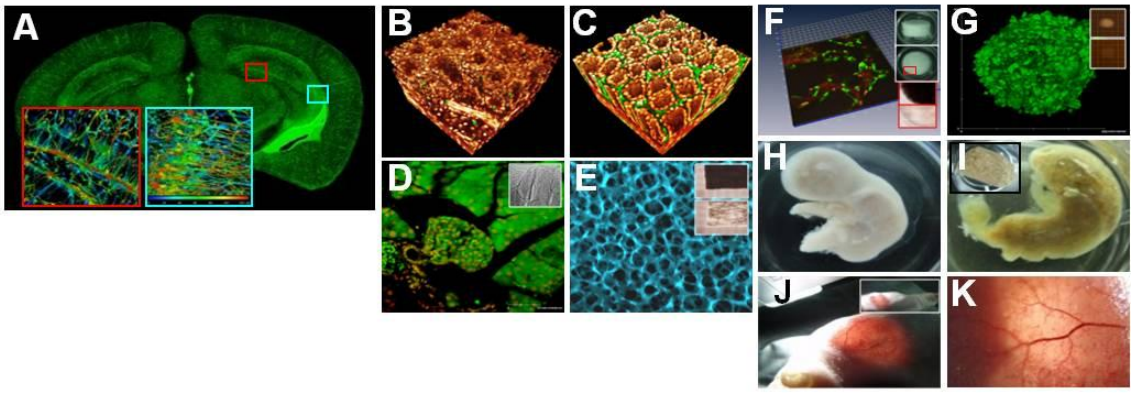
Brain tissues of about 500 micro m thick are derived from an adult female cockroach, Diploptera punctata. (1A) The brain is opaque when incubated in the cockroach physiological saline solution. (1C) The brain becomes completely transparent in FocusClear. The degree of brain transparency is evaluated by the visibility of the underneath colour grids under a dissecting microscope. Bar = 1mm.



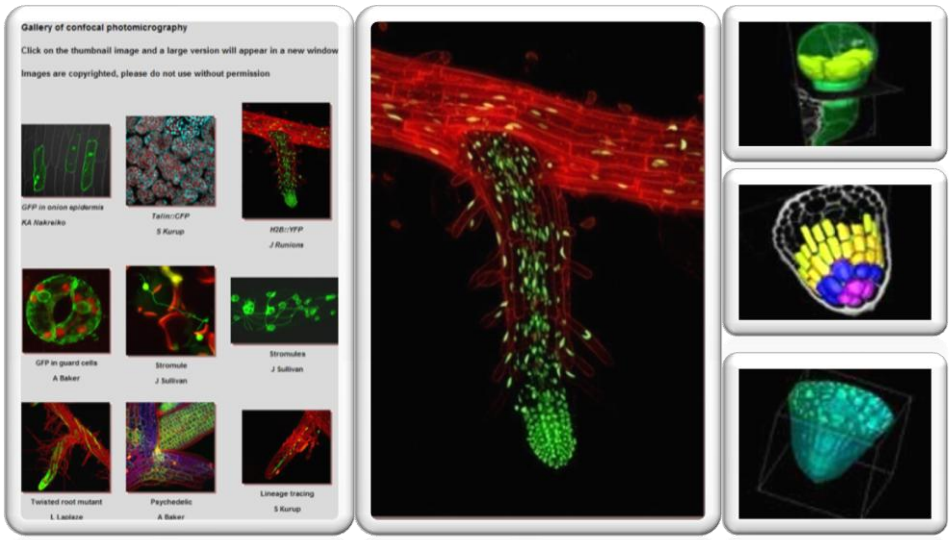
Also, a much clearer fluorescent image and into a much deeper region (over 600 micro m) of the tissue can be observed.

Gallery

Animals



Plants

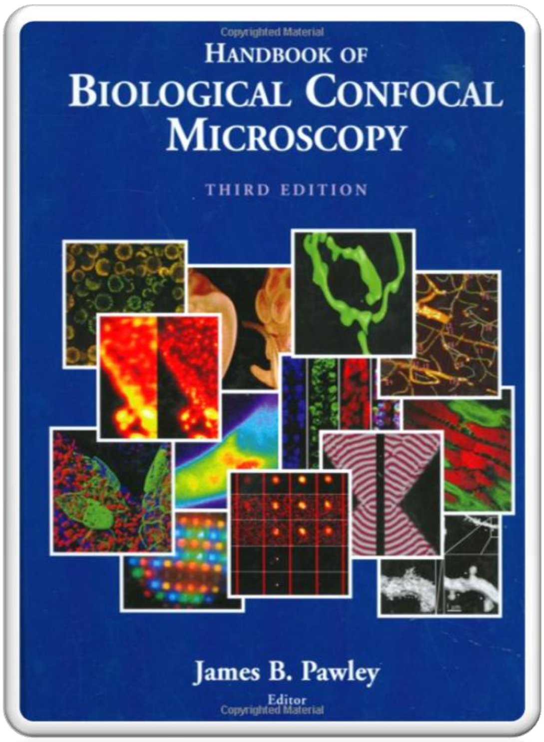


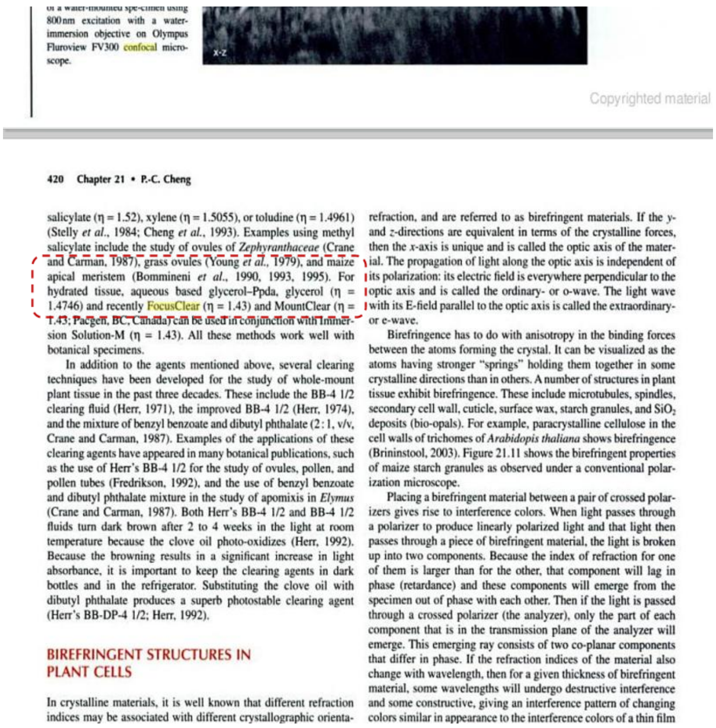
…….FocusClear™ has been used for ultra-high 3D visualisation of insect brains (A.S. Chiang et al., J. Comp. Neurol. 440, 1-11, 2001). We have been using the agents to examine GFP labelled Arabidopsis plants and it has been possible to visualise GFP labelled structures with unprecedented clarity deep within intact tissues…….

Dr. Jim Haseloff Department of Plant Sciences, University of Cambridge

(http://www.plantsci.cam.ac.uk/Haseloff/index.htm)

Handbook





800 nm excitation with a water-immersion objective on Olympus Fluroview FV300 confocal microscope.

salicylate (η = 1.52), xylene (η = 1.5055), or toludine (η = 1.4961) (Stelly *et al.*, 1984; Cheng *et al.*, 1993). Examples using methyl salicylate include the study of ovules of *Zephyranthaceae* (Crane and Carman, 1987), grass ovules (Young *et al.*, 1979), and maize apical meristem (Bommineni *et al.*, 1990, 1993, 1995). For hydrated tissue, aqueous based glycerol–Ppda, glycerol (η = 1.4746) and recently FocusClear (η = 1.43) and MountClear (η = 1.43; Pacgen, BC, Canada) can be used in conjunction with Immersion Solution-M (η = 1.43). All these methods work well with botanical specimens.

In addition to the agents mentioned above, several clearing techniques have been developed for the study of whole-mount plant tissue in the past three decades. These include the BB-4 1/2 clearing fluid (Herr, 1971), the improved BB-4 1/2 (Herr, 1974), and the mixture of benzyl benzoate and dibutyl phthalate (2 : 1, v/v, Crane and Carman, 1987). Examples of the applications of these clearing agents have appeared in many botanical publications, such as the use of Herr's BB-4 1/2 for the study of ovules, pollen, and pollen tubes (Fredrikson, 1992), and the use of benzyl benzoate and dibutyl phthalate mixture in the study of apomixis in *Elymus* (Crane and Carman, 1987). Both Herr's BB-4 1/2 and BB-4 1/2 fluids turn dark brown after 2 to 4 weeks in the light at room temperature because the clove oil photo-oxidizes (Herr, 1992). Because the browning results in a significant increase in light absorbance, it is important to keep the clearing agents in dark bottles and in the refrigerator. Substituting the clove oil with dibutyl phthalate produces a superb photostable clearing agent (Herr's BB-DP-4 1/2; Herr, 1992).

BIREFRINGENT STRUCTURES IN PLANT CELLS

In crystalline materials, it is well known that different refraction indices may be associated with different crystallographic orientations refraction, and are referred to as birefringent materials. If the *y*- and *z*-directions are equivalent in terms of the crystalline forces, then the *x*-axis is unique and is called the optic axis of the material. The propagation of light along the optic axis is independent of its polarization: its electric field is everywhere perpendicular to the optic axis and is called the ordinary- or o-wave. The light wave with its E-field parallel to the optic axis is called the extraordinary- or e-wave.

Birefringence has to do with anisotropy in the binding forces between the atoms forming the crystal. It can be visualized as the atoms having stronger "springs" holding them together in some crystalline directions than in others. A number of structures in plant tissue exhibit birefringence. These include microtubules, spindles, secondary cell wall, cuticle, surface wax, starch granules, and SiO_2 deposits (bio-opals). For example, paracrystalline cellulose in the cell walls of trichomes of *Arabidopis thaliana* shows birefringence (Brininstool, 2003). Figure 21.11 shows the birefringent properties of maize starch granules as observed under a conventional polarization microscope.

Placing a birefringent material between a pair of crossed polarizers gives rise to interference colors. When light passes through a polarizer to produce linearly polarized light and that light then passes through a piece of birefringent material, the light is broken up into two components. Because the index of refraction for one of them is larger than for the other, that component will lag in phase (retardance) and these components will emerge from the specimen out of phase with each other. Then if the light is passed through a crossed polarizer (the analyzer), only the part of each component that is in the transmission plane of the analyzer will emerge. This emerging ray consists of two co-planar components that differ in phase. If the refraction indices of the material also change with wavelength, then for a given thickness of birefringent material, some wavelengths will undergo destructive interference and some constructive, giving an interference pattern of changing colors similar in appearance to the interference colors of a thin film