FluorEver (Catalog #: NBP2-60229)-Protocol

FluorEver is a ready-to-use mounting medium which does not require any further dilutions or mixing with other chemicals. When your cell and tissue samples are ready for coverslipping or/and microscopy analysis follow these steps:

1. Remove the vial with FluorEver from the freezer and warm-it up by keeping for 10-15 minutes at room temperature (this will help to lower the medium's viscosity). It is recommended to avoid medium's exposure to direct light (vial can be placed, for example, into a lab bench drawer during a warm-up step).

   CAUTION: Don't heat FluorEver medium to speed-up its warming because heating may impair its anti-fade activity!

   As soon as a mounting procedure is completed return a vial with FluorEver medium back into a -20°C freezer.
2. Mounting Procedures
   a. Histological Slides:
   After finishing immunohistochemistry or cytochemistyr experiment aspirate the amount of medium needed to cover the entire tissue section or cells on the slide (should be established by investigator, and usually requires 30-40 microliters for covering ~1 cm square of a sample area). Apply FluorEver and then place a coverslip over it. Gently wipe the excess of mounting medium with a soft paper towel. Alternatively, slides can be put vertically on their long side for 2-5 minutes to drain the excess of the mounting medium. Dispense FluorEver with easier disposable transfer pipets or glass Pasteur pipettes (Alternatively, plastic pipette tips for repeat pipetors can be trimmed with either scissors or a razor blade to have a wider opening for easier aspiration of the FluorEver mounting medium from the vial and dispensing it over histological samples.
   b. Multi-well culture plates:
   After fixing (e.g. formaldehyde, acetone, ethanole, etc.) and washing cells with buffer of choice (e.g. PBS, TRIS, etc) add FluorEver medium directly into wells so that cells are completely covered with the mounting medium. Refer to the table below to determine the amount of mounting medium depending on the format of the plate.

96-well plate	50 microliters/well
24-well plate	200-300 microliters/well
12-well plate	400-500 microliters/well
6-well plate	1-1.3 ml

3. Now samples are ready for a long-term storage and for fluorescence and confocal microscopy examination.
4. FluorEver mounted samples need to be stored in a horizontal position at -20°C or below. Histological slides can be stored, for example, in slide trays. Samples in culture plates also need to be stored at -20°C or below in a horizontal position (it is recommended to cover culture plates with aluminum foil for light protection).

Stability: If stored and used properly (see below) FluorEver fully retains its fluorescence protecting properties for at least six months after opening the vial.

Storage: Store at -20°C and protect from exposure to direct light.

Physical appearance: Clear liquid, slightly viscous at room temperature. Viscosity increases with temperature decrease. Very viscous at -20°C (storage temperature).

Safety: Avoid contact of FluorEver with eyes and skin. Don't aspirate FluorEver using mouth-controlled pipettes. Wear gloves and a lab coat when working with FluorEver mounting medium.

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