Cellorama®

CellO®-IF3 : All-in-One Labeling Reagent for Organoids & Spheroids

Label All, Analyze All: Unparalleled Accuracy in Spheroid/Organoid Analysis

Product Description:

CellO®-IF3 is an all-in-one reagent specifically designed for whole-mount immunofluorescent staining of organoids and spheroids. The technology enables labeling 3D samples while still in hydrogel or extracellular matrix.

Key Features:

- **Efficiency:** Reduces the number of staining steps and saves time.
- **Convenience:** Simplifies the workflow and minimizes the risk of errors.
- **Versatility:** Allows for the simultaneous analysis of multiple markers within the same sample.
- **High Quality Images:** Boosts antigenicity for clearer visualization of target and reduces background
- **Simplicity:** Eliminates separate harvesting, clearing, antigen retrieval, permeabilization, blocking steps

Content: CellO®-IF3, All-in-One Reagent, Ready-to-Use

- For Research Use Only
- Not for use in diagnostic procedures

Amount:

- Catalog No. 248321 15 mL
- Catalog No. 248322 45 mL
- Catalog No. 248324 90 mL

Storage: Store at 2-8°C, Keep in dark

- Avoid thawing cycles of CellO®-IF3, and warm only the needed amount at each experiment.
- Avoid contamination.

Expiration Date:

- The shelf life is 1 year from the date of manufacture.
- The expiration date is printed on the item.

Caution:

- Wear appropriate protective eyewear, clothing, and gloves.
- Read the Safety Data Sheet (SDS) and follow the handling instructions.
- In the event of accidental ingestion or contact with the eyes, immediately wash the effected area and seek medical attention.

Immunofluorescent Labeling Protocol:

Materials Needed :

CellO®-IF3 / PBS / Primary Antibody / Secondary Antibody

Protocol:

- Solutions are warmed prior to the experiment at 37°C.
- Grow cells in a culture vessel with high optical quality
- Aspirate growth medium
- Leave the sample on ice for 2 min to reduce the hydrogel's thickness and improve reagent penetration. (This step is omitted if the sample is not embedded in a hydrogel or if the hydrogel layer is minimal.)
- Fix in 4% paraformaldehyde (PFA) for 15 min- 1hr

All following steps are performed at 37°C:

- Remove the fixative and wash with CellO®-IF3, X3, 10 min
- Incubate for 1-2 hr at 37 C with primary antibody diluted in CellO®-IF3
- Wash with CellO®-IF3, X3, 10 min
- Incubate for 1–2 hr with secondary antibody diluted in CellO®-IF3
- Wash with PBS, X3, 10 min
- Mount & Image under a microscope

Technical Note:

- The protocol is compatible with various cell culture vessels with high optical quality bottoms, like coverslips, micro-chambered slides, glass-bottomed dishes, and CellO-M dishes.
- A low-speed orbital shaker at every step or a shaking incubator at 37°C may facilitate labeling and even staining.
- DO NOT leave samples in CellO®-IF3 longer than recommended.
- While smaller hydrogel domes often result in better reagent penetration and brighter images, larger domes can be successfully labeled with a slight adjustment. For larger volumes, chilling the sample on ice for 2 minutes before fixation can help reduce the hydrogel's thickness and improve reagent penetration.
- Multiple Labeling: Primary antibodies are diluted in the same CellO®-IF3. Similarly, the corresponding secondary antibodies are also diluted in the same reagent. Selecting the appropriate secondary antibodies is crucial to prevent overlapping signals and ensure accurate staining.
- Glycerol or commercially available aqueous mounting media are suitable for mounting labeled samples.
- Formulation of mounting medium for nuclear labeling:
  1,2 uL Hoechst or DAPI+675 uL PBS + 675 uL Glycerol

References:

(1) Demirel, G., Tok, O.E., Aktas, R.G. (2025). A Simplified and Robust Immunofluorescence Labeling Method for Complex 3D Cell Cultures: Minimizing Manipulation and Maximizing Data in Whole-Mount Analysis of Organoids, Spheroids, and Co-culture Models. In: Methods in Molecular Biology. Springer, New York, NY. https://doi.org/10.1007/7651_2025_634

(2) Demirel, G., Cakıl, Y.D., Koltuk, G. *et al*. (2024). The use of hyaluronic acid in a 3D biomimetic scaffold supports spheroid formation and the culture of cancer stem cells. *Sci Rep* 14, 19560. https://doi.org/10.1038/s41598-024-69047-6

(3) Tok, O. E., Demirel, G., Saatci, Y., Akbulut, Z., Kayalar, O., Aktas, R. G. (2022). Culturing, Freezing, Processing, and Imaging of Entire Organoids and Spheroids While Still in a Hydrogel. J. Vis. Exp. (190), e64563, doi:10.3791/64563