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Cell*Cover*

Ready to use fixative for immediate protection of biomolecules and cellular morphology.

For research use only.

The Product

Product description: Non-toxic, ready to use solution for protecting DNA, RNA and protein as well as morphology in human and animal cells and tissues. Cell*Cover* allows parallel storage of biomolecules within their cellular context without chemical crosslinking.

How it works: Cell*Cover* was developed for fast "one step" stabilizing biomolecules with simultaneous maintenance of morphology of tissue specimens and cultured cells. Cell*Cover* exerts its effect by shielding molecules against enzymatic hydrolysis and protection of cellular integrity. Thus, cells and tissue treated with Cell*Cover* maintain a close to native state. They stabilize DNA, RNA, protein and morphology, allowing modern sophisticated analyses of genomics, transcriptomics and proteomics.

Protecting DNA, RNA and protein in tissue

Procedure: Tissue should be fresh. Tissue cube edge should be less then 0,5 cm. The volume of Cell*Cover* added must be at least 10x specimen volume. Change Cell*Cover* at least once after 4-24 hours.

Cell*Cover* must be cooled to 4°C before before use. Store specimen in Cell*Cover* at 4°C. Proceed according to experimental design e.g. RNA isolation

Cell*Cover* protects biomolecules up to two weeks at 4°C. If native structure of protein is of minor importance (e. g. SDS-PAGE), samples might be frozen.

Notes:

Freezing specimen in Cell*Cover* might destroy RNA and morphology upon thawing.

Capsuled organs: When protecting biomolecules in capsuled organs (e. g. kidney), capsule must be opened up (e.g. divide tissue at least once).

Possible downstream applications:

- ISH: in situ hybridization (RNA as well as DNA FISH and CISH!)

- Batch/ single cell transcriptome analysis
- Flow Cytometry
- *Many more applications*

Protecting DNA, RNA and protein in cultured cells

Procedure: Use freshly harvested cells, centrifuge between 600g and 1200g (depending on cell type) poor off SN and resuspend cells in 10 volumes cold Cell*Cover (Optional: centrifuge cells again, remove SN and resuspend cells in fresh CellCover).* Store at 4°C until use. Covered cells should not be left at RT.

Cell*Cover* protects protein up to two weeks at 4°C. If native structure of protein is of minor importance (e. g. SDS-PAGE), samples might be frozen.

Notes:

If cells contain lot of debris, wash cells twice in appropriate buffer or medium.

Isolating protected biomolecules

Protected Biomolecules can be isolated by any procedure.

Procedure: If sample is in isopropanol rehydrate by washing cells with 3 changes of ice-cold Cell*Cover*.

Cells: centrifuge at 4°C, remove SN as good as possible, proceed with your protocol or with manufacturers recommendation if using a kit. Cell*Cover* is compatible with commonly used detergent for cell lysis

Tissue: remove tissue block from Cell*Cover* and place in a fresh tube, proceed with your protocol or with manufacturers recommendation, if using a kit.

Notes:

If there is need to spin down cells after thawing, use a pre-cooled centrifuge.

If protein is to be isolated out of tissue blocks, mince tissue on ice before proceeding with protein isolation.

Cell*Cover* is not compatible with EDTA.

Overview of related protocols (use-cases)

Procedure: Cell*Cover* exerts its stabilizing effect almost instantly. It allows the parallel storage of DNA, RNA and proteins within it's cellular context while maintaining cells morpholohy (no crosslinking activity)! The following provides you with an overview of related protocols for some typical and special applications for which Cell*Cover* shall be the basic reagent for preparation of your specimens:

Fixation on TCP (adherent cells)

1. Seed and grow cells until confluence (on chamber slides)
2. Remove medium
3. Optional: Wash cells 1x with (D)PBS or Cell*Cover*
4. Add cold Cell*Cover* according to surface area
5. Optional: Remove CellCover and replace with fresh cold Cell*Cover*
6. Store at 4°C until needed or proceed to staining protocol according to experimental design, e.g. immunostaining
   If RNA is to be isolated for downstream application, you can stain cells by using CellCover as antibody diluent and washing buffer.

Notes:

Attachment of cells to the substrate is critical. Cell*Cover* does not have crosslinking properties. If you work with cells just loosely attaching to the slide, cells might float off the substrate. You can try following workarounds:

- Coat substrate, best coating must be found experimentally
- Dip in H2O, drain slide and dry sample

Possible downstream applications:

- Laser capture microscopy
- ISH: in situ hybridization (RNA as well as DNA FISH and CISH!)
- Batch/ single cell transcriptome analysis
- *Many more applications*

Fixation of suspension cells

1. Harvest cells by centrifugation
2. Remove supernatant
3. Resuspend cells in cold Cell*Cover* (at least 10x volumes)
4. Add Cell*Cover* (5 to 10x volumes) and
5. Optional: pellet cells by centrifugation and resuspend in fresh cold Cell*Cover*
6. Store at 4°C until needed or proceed to standard protocols, e.g. immunostaining
   If RNA is to be isolated for downstream application, you can stain cells by using CellCover as antibody diluent and washing buffer.

Notes:

Cell*Cover* is not compatible with EDTA

Possible downstream applications:

- Flow cytometry/ flow cytometric sorting
- Batch / single cell transcriptome analyses

- Single cell sequencing
- *Many more applications*

Special – Sticking to scheduled time points when finishing and working up cell culture experiments – Generate suspension of cells from adherent Cell*Cover* fixed cells:

1. Remove media
2. Optional: wash cells 1x with (D)PBS
3. Add appropriate volume of cold Cell*Cover* according to surface area
4. Incubate cells for 2-5 minutes at RT
5. Remove Cell*Cover*
6. Detach cells (e.g. Trypsin/EDTA 0,25% at 37°C)
7. Centrifuge cells
8. Wash cells once with (D)PBS and centrifuge again
9. Remove PBS and resuspend cells in cold CellCover (10x volumes)
10. Store at 4°C until needed or proceed with your (downstream) application(s)

Possible downstream applications:

- Batch and single cell analysis
- Batch and single cell transcriptome analysis
- Immunocytochemistry
- Immunohistochemistry
- DNA/ RNA/ Protein Isolation
- ISH (RNA as well as DNA FISH and CISH)
- Laser capture mircroscopy
- Microarray analysis
- NGS
- PCR
- RNA Sequencing
- Northern Blotting
- Western Blotting
- *Many more applications*

Notes: Appropriate volume of CellCover

TCP	CellCover volume
96-well	100µL
48-well	300µL
24-well	500µL
12-well	1mL
6-well	2mL
10cm dish	4mL

Cell culture flasks	CellCover volume
T12,5	1mL
T25	2,5mL
T75	8mL
T175	15mL

For special applications or further questions please read our FAQ on www.anacyte.com or please contact us at support@anacyte.com