Store at 4°C and -20°C

Cell Fractionation Kit (Eco-friendly detergent)

#42193

1 Kit
(20 assays)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Kit Quantity	Storage Temp
Cytoplasmic Isolation Buffer (CIB)	9041	1 x 10 mL	-20°C
Membrane Isolation Buffer, Eco-friendly (MIB)	67356	1 x 10 mL	-20°C
Cytoskeletal/Nuclear Isolation Buffer (CyNIB)	9049	1 x 5 mL	-20°C
Protease Inhibitor Cocktail (100X)	5871	1 x 250 µL	4°C

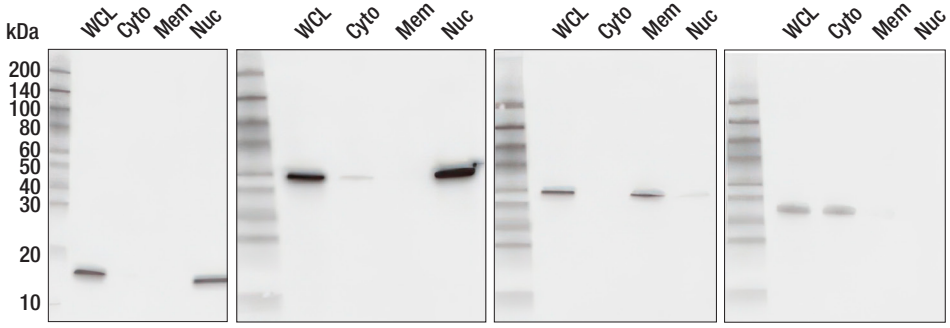
Description: The Cell Fractionation Kit is designed to provide a fast and efficient way of separating cultured cells into three distinct fractions: cytoplasmic, membrane/organelle, and nuclear/cytoskeletal. These fractions can then be analyzed by SDS-PAGE and western blotting. The kit includes enough buffer for 20 assays.

This kit uses ECOSURF SA-9, a non-toxic, biodegradable, non-ionic alkylpolyglucoside-based surfactant, in place of non-ionic surfactant Triton X-100 in the CST Cell Fractionation Kit #9038. Use of Triton X-100 use is being phased out of many laboratory settings due to concerns about toxicity and environmental impact. ECOSURF SA-9 is derived from seed oil and follows the "Design for Degradation" principle of Green Chemistry, thus providing a more environmentally safe option.

ECOSURF SA-9 has comparable surfactant properties to Triton X-100, it does not react with charged molecules (proteins, nucleic acids), and it is stable over a wide pH range, making it a suitable replacement for many biological applications including cell fractionation. This kit performs equally as well as the original Triton-based kit.

Background: Cellular fractionation allows for the extraction of cellular proteins into distinct compartments. This is achieved by the use of detergents that take advantage of the inherent qualities and composition of different cellular membranes (1). Cellular fractionation has been important for defining the localization of many proteins, observing the translocation of proteins, and determining protein-protein complexes, such as cytoskeletal-associated proteins (2,3). Thus, detergent-based cellular fractionation separates cellular components with greater ease and speed compared to a more laborious density centrifugation method (4).

Specificity/Sensitivity: The Cell Fractionation Kit (Eco-friendly detergent) is intended to be used with cultured cell lines that are either adherent or in suspension. This kit allows for the determination of the subcellular localization of proteins through the separation into three distinct fractions. Separation efficiency can be measured using the Cell Signaling Technology® Cell Fractionation Antibody Sampler Kit #11843.



Western blot analysis of cell fractions from HeLa cells using Histone H3 (D1H2) XP® Rabbit mAb #4499, Vimentin (D21H3) XP® Rabbit mAb #5741, PDI (C81H6) Rabbit mAb #3501, and MEK1/2 (D1A5) Rabbit mAb #8727 showing cytoplasmic, organellular/membrane, and nuclear/cytoskeletal localization. Whole cell lysates (WCL) were used to represent total protein. Cytoplasmic proteins (Cyto) were isolated using CIB buffer. Integral membrane and organellular proteins (Mem) were isolated using MIB buffer. Nuclear and cytoskeletal proteins (Nuc) were isolated using CyNIB buffer.

Storage: Store at -20ºC. Upon receipt, Protease Inhibitor Cocktail (100X) #5871 should be stored at 4ºC. All other components should be stored at -20ºC.

Please visit cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Lenstra, J.A. and Bloemendal, H. (1983) *Eur J Biochem* 135, 413-23.

(2) Banno, A. et al. (2012) *J Biol Chem* 287, 13799-812.

(3) Loo, L.H. et al. (2009) *J Cell Biol* 187, 375-84.

(4) Michelsen, U. and von Hagen, J. (2009) *Methods Enzymol* 463, 305-28.

U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Cell Fractionation Kit Protocol

A. Buffers

- **Cytoplasmic Isolation Buffer (CIB) #9041** – 10 mL, Store at -20°C.
- **EcoSafeSurf EH-9 Membrane Isolation Buffer (MIB) #67356** – 10 mL, Store at -20°C.
- **Cytoskeletal/Nuclear Isolation Buffer (CyNIB) #9049** – 5 mL, Store at -20°C.
- **Protease Inhibitor Cocktail (100X) (#5871)** – 250 µL, Store at 4°C.

B. Notes

- All steps, except for the addition and sonication of the CyNIB buffer, should be done on ice or at 4°C.
- Adherent or suspension cultured cells can be used for this assay.
- 1X protease inhibitors (#5871) (5 µL of 100X per 500 µL buffer, included in kit) and 1 mM fresh PMSF (#8553) (2.5 µL of 200 mM PMSF per 500 µL buffer, not included in kit) should be added to each buffer immediately before use.
- Phosphatase inhibitors are already included in the buffers. There is no need to add them.
- Please refer to Table 1 for the appropriate volumes for your specific cell concentration.
- The volumes given in Sections D and E are based on cell counts obtained from HeLa cells at ~90% confluency in a 10 cm cell culture dish (5 x 10^6 cells).
- If the CyNIB buffer is cloudy after thawing, please warm the solution in a 37°C water bath until the solution is clear.
- Be cautious when saving fractions so that you do not get any contamination from the resulting pellet.
- All lysates should be stored at -20°C for short term storage (less than 1 month) or -80°C for long term storage (greater than 1 month).

C. Isolating Cell Population

For adherent cells

1. Wash plate with cold 1X PBS.
2. Trypsinize the plate.
3. Add cold growth media to deactivate trypsin.

For both adherent and suspension cells

1. Spin down cells at 350 x g for 5 min.
2. Aspirate media.
3. Wash cell pellet with cold 1X PBS.
4. Resuspend pellet in 0.5 mL of cold 1X PBS.
5. Count live cells using Trypan Blue and a hemacytometer.

D. Detection of Proteins

1. Aliquot 100 µL of cell suspension into a 1.5 mL tube for the whole cell lysate (WCL).
2. Add 60 µL of 3X SDS Loading Buffer with DTT (#7722) to make a final volume of 160 µL of WCL.
3. Sonicate WCL tube for 15 sec at 20% power 3 times, heat for 5 min at 95°C, and centrifuge for 3 min at 15,000 x g.

E. Cell Fractionation

1. Aliquot the remaining 400 µL into a 1.5 mL tube.
2. Centrifuge for 5 min at 500 x g at 4°C.
3. Aspirate the supernatant.
4. Resuspend pellet in **500 µL of CIB**.
5. Vortex for 5 sec.
6. Incubate on ice for 5 min.
7. Centrifuge for 5 min at 500 x g.
8. Save the supernatant. This is the **Cytoplasmic Fraction**.
9. Resuspend pellet in **500 µL of MIB**.
10. Vortex for 15 sec.
11. Incubate on ice for 5 min.
12. Centrifuge for 5 min at 8,000 x g.
13. Save the supernatant. This is the **Membrane and Organelle Fraction**.
14. Resuspend pellet in **250 µL of CyNIB**.
15. Sonicate for 5 sec at 20% power 3 times. This is the **Cytoskeletal and Nuclear Fraction**.

F. Western Blot

1. Add 60 µL of 3X SDS Loading Buffer with DTT (#7722) for every 100 µL of supernatant.
2. Boil each sample for 5 min at 95°C and centrifuge for 3 min at 15,000 x g.
3. Load 15 µL of each fraction along with 15 µL of WCL.

Table 1: Volumes in µL for WCL or buffer at indicated cell numbers.

	Cell Count			
	2.5 x 10^6 cells	**5 x 10^6 cells**	**7.5 x 10^6 cells**	**1 x 10^7 cells**
WCL	50	100	150	200
CIB	250	500	750	1000
MIB	250	500	750	1000
CyNIB	125	250	375	500

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