Minute™ Organic Solvent-Free Milk Lipid Depletion Kit

Cat. No. ML-044

Description

Human/animal whole milk contain large amount of lipids that should be removed prior to milk protein analysis and purification. Traditionally, organic solvent extraction such as the Folch method is the method of choice. The use of chloroform as organic phase is undesirable due to its toxicity. Many organic solvents can effectively extract lipid from milk but also precipitate proteins that become insoluble upon resuspension. With this kit milk proteins are precipitated in an aqueous environment and can be easily re-dissolved in aqueous buffer systems for downstream applications. The kit can separate whole milk into three fractions: water soluble protein fraction, casein fraction and lipid fraction without using organic solvents. The extracted lipid fraction can be used directly or resuspended in organic solvent of your choice then dried for downstream lipid analysis. Each kit provides sufficient reagents for up to 100 preps based on a 100 µL sample size. The protocol can be scaled up or down depends upon researcher's need.

Applications

This kit can deplete majority of lipids from milk without heat-treatment to facilitate protein analysis or protein purification. The separated lipid fraction can also be used for further analysis such as lipidomic studies.

Kit Components

Buffer A	10 ml
Buffer B	20 ml

Additional reagents and equipment required

1 X PBS, Table top centrifuge and Eppendorf tubes

Shipping and Storage: Ship at ambient temperature and stored at room temperature

Protocol

Important: Shake buffer B bottle vigorously for a few seconds prior to use.

1. Add milk sample to a test tube. For demonstration purpose, we recommend to add 100 µl whole milk to a 1.5 ml Eppendorf tube.
2. Add equal amount (100 µl) of buffer A to the tube and mix by pipetting up and down for 10-20 times. Incubate the tube on ice for 10 min.
3. Centrifuge at 16,000 X g at room temperature for 5 min. Transfer the supernatant to a fresh Eppendorf tube. The supernatant contains mainly lactoglobulin and lactalbumin (La fraction).
4. Resuspend the pellet from step 3 in 100 µl PBS by vortexing**. Shake buffer B bottle vigorously for about 10 second**s and immediately transfer 200 ul buffer B to the tube. Mix well by vortexing vigorously for 20 second and incubate at room temperature for 2-3 min.

5. Centrifuge the tube at 16,000 X g for 5 min at room temperature. After centrifugation, a lipid phase (upper portion) and a pellet (casein fraction) should be clearly visible.
6. Pour aqueous and lipid phases into a fresh 1.5 ml microfuge tube. Wipe out the wall of the tube with a small piece of paper towel using forceps to remove lipids attached to the wall. The pellet contains mainly casein and other proteins with majority of lipids depleted.
7. The pellet can be resuspended in an aqueous buffer such as 200 µl PBS. The casein proteins are readily dissolved in the buffer. The residual lipids can be further removed by centrifuging the tube at 10,000 X g for 5 min. Remove the white thin lipid layer on top. If one is interested in milk lipids, the isolated lipids can be further extracted using organic solvents such as hexane/isopropanol mixture extraction (Anal Biochem 1978,90,420) for downstream analysis.

   Note: If you are working with expressed proteins in the milk. You should check the presence of your target protein in both La and casein fractions.