

Cell Fractionation Kit

Lysis Buffer

Order No.: 3002-0150/CF-Kit
Lot: 3002B
Size (ml) 3 x 50 ml



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01/300707F

Formulation

The buffers CF-CYT, CF-MEM and CF-NE contain phosphatase inhibitors. 0.09% NaN₃ and *Antifreeze* solution that avoids precipitation of buffer components after a freeze/thaw cycle.

Before use add protease inhibitors to the lysis buffer, e.g. Protease inhibitor cocktail tablets (Cat. No.: 11 69 498 001), Roche Diagnostic GmbH, Mannheim, Germany.

Storage

For long term storage, freeze buffer (-20°) upon arrival. Thawed buffer may be stored at 4°C up to 4 weeks.

Application

Before use lysis buffer that was stored at 4°C must be incubated at 37°C to solve the detergent. After this allow the buffer to reach room temperature before cell lysis. Adherent cells should be washed once with serum- and additive - free cell culture medium.

Use 1 ml lysis buffer CF-CYT per petridish of 100mm diameter. Perform cell lysis at room temperature under optical control until completed. Clear spin (5 min, 1000xg, 4°C). Recover supernatant (= Cytoplasma), carefully resuspend pellet with buffer CF-CYT and process as above. Resuspend pellet in 1ml buffer CF-MEM and incubate 5min at room temperature. Clear spin (5min at 2000xg) and recover supernatant (membrane fraction). Wash the pellet once with buffer CF-MEM and resuspend in buffer CF-NE. Incubate 5 min at room temperature. Clear spin (5min, 12.000xg) and recover supernatant (nuclear fraction).

Freeze the fractions in liquid nitrogen or add SDS-PAGE sample buffer and process for SDS - PAGE and immunoblotting.