

Chaps Cell Extract Buffer

Supplied as a 10X stock

5 ml



Cell Signaling
TECHNOLOGY®

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Description: Chaps Cell Extract Buffer can be used to lyse cells under non-denaturing conditions and is recommended for the preparation of cell lysates to be used with our caspase signaling pathway antibodies (see "Companion Products").

Components:

10X Chaps Cell Extract Buffer (5 ml)

1X concentration:

50 mM Pipes/HCl (pH 6.5), 2 mM EDTA, 0.1% Chaps, 20 µg/ml Leupeptin, 10 µg/ml Pepstatin A, 10 µg/ml Aprotinin

200X DTT (0.25 ml)

1X concentration:

5 mM DTT

Preparation of 1X Cell Extract Buffer:

- Buffer should be prepared immediately before use.
- Immediately before dilution, invert the 10X Chaps Cell Extract Buffer several times to suspend all buffer components.
- Add 1/10 volume of 10X Chaps Cell Extract Buffer to 9/10 volume of Milli-Q water or equivalently purified water.
- Add DTT to final concentration of 5 mM (1:200 dilution) and PMSF* to final concentration of 1 mM.

*reagent not included

Sample Preparation Using Chaps Cell Extract Buffer:

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells three times with PBS; aspirate. Scrape cells into PBS and spin down to pellet.
- Lyse cells by adding Chaps Cell Extract Buffer (1 volume of cell pellet). Resuspend cells in the buffer, freeze and thaw three times, centrifuge lysate at 14,000 rpm. Keep the supernatant and discard the pelleted cell debris.
- Add SDS Sample Buffer and heat sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 5 µl onto SDS-PAGE gel (10 cm x 10 cm).

Storage: Store Chaps Cell Extract Buffer and dithiothreitol (DTT) at -20°C.

Companion Products:

Cleaved Caspase-7 (Asp198) Antibody #9491

Caspase-7 Antibody #9492

Cleaved Caspase-9 (Asp330) Antibody #9501

Caspase-9 Antibody #9502

Cleaved Caspase-9 (Asp315) Antibody #9505

Cleaved Caspase-3 (Asp175) Antibody #9661

Caspase-3 Antibody #9662

Caspase-10 Antibody #9752

Caspase-6 Antibody #9762

Caspase-7 Activity Assay Kit #9850