

## Lysate Preparation

Calyculin A is a serine/threonine phosphatase inhibitor that inhibits the activity of protein phosphatases PP1 and PP2A. Cells treated with calyculin A for 30 minutes can undergo significant threonine phosphorylation, as shown by western blotting using anti-Phosphoserine/threonine, cat.# PP2551, as compared to untreated, control cell lysates.

C2C12 is a mouse muscle myoblast cell line that is useful for studies of myoblast and osteoblast differentiation. Confluent cultures of C2C12 cells were serum starved for 2 hours. Cells were then either left untreated (Cat.# CL9511) or treated with Calyculin A (100 nM) for 30 minutes at 37°C (cat.# CL9521). Cells were lysed in 1% SDS, 1.0 mM sodium orthovanadate, 1 mM sodium fluoride in 10 mM Tris (pH 7.4) buffer. Protein concentration was determined using the BCA method (Pierce) before diluting to final concentration and buffer.

## Buffer and Storage

Cell Lysates are supplied at a concentration of 1 mg/ml in electrophoresis sample buffer (62.5 mM Tris pH 6.8, 2% SDS, 5% glycerol, 0.003% bromophenol blue, 0.9%  $\beta$ -mercaptoethanol). Store at  $-20^{\circ}\text{C}$ . Do not boil or dilute. Stable for 1 year.

## Applications

WB                    20  $\mu$ l/lane

End user should determine optimal quantity for their particular applications and experiments.

## Related Products

CL8021	C2C12 Lysate
CL9511	C2C12 Calyculin A Control Lysate
CL8031	C2C12 Control Lysate
CL8041	C2C12 + Pervanadate Lysate
ML7051	Mouse Muscle Lysate

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