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pTyr control lysate

Description

The phospho-tyrosine (pTyr) control lysate was generated from transfected HEK293 cells, expressing the viral protein-tyrosine kinase v-Src.
This virus-derived kinase is constitutively active and phosphorylates several cellular proteins, even in serum-starved cells. In contrast, tyrosine phosphorylation of cellular proteins in cells transfected with the empty vector (mock lysate) is barely detectable under these conditions. The cell-lysates were prepared using a lysis buffer, which contains multiple phosphatase-inhibitors to preserve the protein-tyrosine phosphorylation in the cell lysates.
In lysates from v-Src expressing cells (pTyr control lysate) cellular proteins with an apparent molecular weight of ~30, 55, and 75 kDa are the most intense bands detectable by Western Blotting with the anti-pTyr antibody.

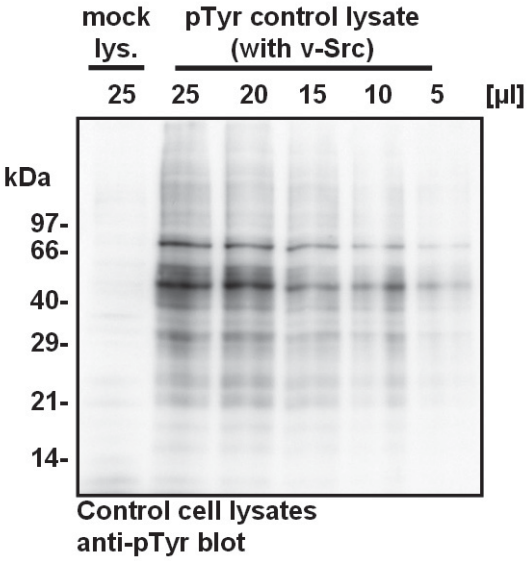
Product

Whole cell lysate:
50 µl
Cell line:
HEK293 cells
Overexpressed protein:
v-Src , functioning as a constitutively-active protein-tyrosine kinase
MW:
multiple pTyr proteins (~30, 55, and 75 kDa)
Lysis buffer:
60 mM Tris/HCL pH 6.8, 2% SDS,
5% mercaptoethanol,
50 µg/ml bromophenolblue, 10% glycerine
Application:
For SDS-PAGE/Western Blotting
20 µl of the lysates should be loaded per lane of a 10% polyacrylamide-gel.

Shipping and storage

Shipping:
The lysate is shipped in cold case.
Storage:
Lysate is stable for 1 week at 4°C.
For prolonged storage, the lysate should be stored at -80°C. Aliquot to avoid repeated freezing and thawing.
At -80°C, the product is stable for at least 1 year from shipment.

Application in quality control



Control cell lysates
anti-pTyr blot

Western Blotting using the pTyr control lysate and the mock lysate.
HEK293 cells were transiently transfected either with a eukaryotic expression vector, encoding the cDNA for the protein tyrosine kinase v-Src (pTyr control lysate), or with the empty expression vector (mock lysate; mock lys.).
The indicated amounts of the pTyr control-lysate (25, 20, 15, 10, or 5 µl) or 25 µl of the mock lysate, respectively, were separated by SDS-PAGE on a 10% polyacrylamide-gel and then transfered onto a PVDF membrane. The membrane was probed with the monoclonal anti-pTyr antibody from *tag*-tools (clone TT11; dilution 1:1.000).
The bound antibody was detected by incubation with HRP-coupled protein G (dilution 1:10.000) and visualized using chemiluminescence. The X-ray film was exposed for 15 seconds.
Multiple tyrosine phosphorylated proteins are visible in 5 - 25 µl of the lysates from v-Src expressing cells (pTyr control lysate), with the most prominent bands at ~30, 55, and 75 kDa. In contrast, barely any tyrosine phosphorylation is detectable in 25 µl of the mock lysate derived from control transfected, serum-starved cells.

Use

The pTyr control lysate and the mock lysate are for quality control in research applications only. They are not intended for diagnostic or therapeutic purposes.