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FIVE-tag control-lysate

Description

The FIVE-tag control-lysate was generated from transfected HEK293 cells, expressing a FIVE-tagged protein. The FIVE-tag corresponds to a pentapeptide (PEYFK) of the Nef protein from the HI virus and is one of the most compact epitope-tags in use.
To generate the FIVE-tag control-lysate, HEK293 cells were transiently transfected with a mammalian expression vector coding for a cytoplasmic protein harbouring an amino-terminal FIVE-tag. A whole cell lysate was prepared from these transiently transfected cells. Besides all other cellular proteins, this whole cell lysate contains the FIVE-tag protein, which shows an apparent molecular weight of ~70 kDa upon SDS-PAGE.

In addition to the FIVE-tag control-lysate, a mock-lysate was prepared from HEK293 cells, which were transfected with the empty expression vector.

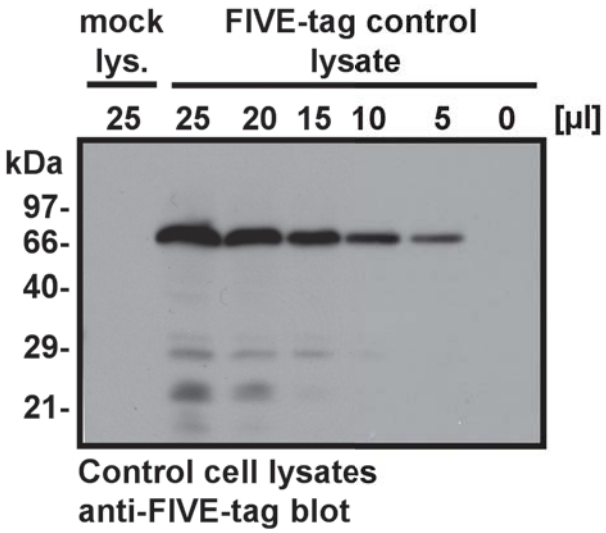
Product

Whole cell lysate:
50 µl
Cell line:
HEK293 cells
Tagged protein:
Human cytoplasmic protein
with an amino-terminal FIVE-tag.
MW:
~70 kDa
Lysis buffer:
60 mM Tris/HCL pH 6.8, 2% SDS,
5% mercaptoethanol,
50 µg/ml bromophenolblue, 10% glycerol
Application:
For SDS-PAGE/Western Blotting
15 µl of the lysates should be applied per lane of a 15% 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-FIVE-tag blot

Western Blotting using the FIVE-tag control-lysate and the mock-lysate.
HEK293 cells were transiently transfected either with the empty expression vector (mock-lysate; mock lys.) or with an expression vector encompassing the cDNA of a FIVE-tag protein (FIVE-tag control-lysate).
25 µl of the mock-lysate and the indicated amounts of the FIVE-tag control-lysate (25, 20, 15, 10, 5, or 0 µl) were separated by SDS-PAGE using a 15% polyacrylamide-gel and then transfered onto a PVDF membrane. The membrane was probed with the monoclonal anti-FIVE-tag antibody from *tag*-tools (clone TT1; dilution 1:2,000).
The FIVE-tag bound primary 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 30 seconds.
Whereas a specific ~70 kDa band is detected in the FIVE-tag control-lysate at all applied volumes (5 - 25 µl), no binding of the anti-FIVE-tag antibody to cellular proteins can be detected in the lane, where 25 µl of the mock-lysate was applied.
At larger volumes of the FIVE-tag control-lysate (20 and 25 µl), additional protein bands are detected, which comprise degradation products of the FIVE-tag protein.

Use

The FIVE-tag control-lysate and the mock-lysate are for quality control in research applications only. Not for diagnostic or therapeutic purpose.