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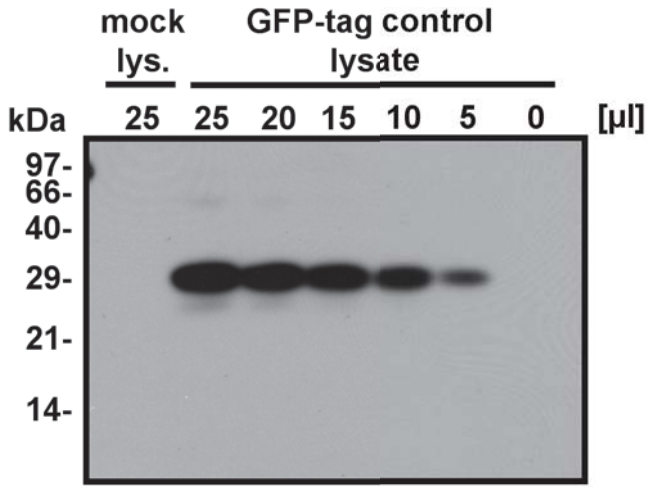
GFP-tag control-lysate

Description

To generate the GFP-tag control-lysate HEK293 cells were transiently transfected with a mammalian expression vector coding for the green fluorescent protein (GFP) from the jelly fish *Aequorea victoria*. A whole cell lysate was prepared from these transiently transfected cells.
Besides all other cellular proteins, this whole cell lysate contains the GFP protein, which shows an apparent molecular weight of ~28 kDa upon SDS-PAGE.

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

Application in quality control



Control cell lysates
anti-GFP-tag blot

Western Blotting using the GFP-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 green fluorescent protein (GFP-tag control-lysate). 25 µl of the mock-lysate and the indicated amounts of the GFP-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 polyclonal anti-GFP-tag antibody from *tag*-tools (dilution 1:500).
The GFP-tag bound primary antibody was detected by incubation with HRP-coupled protein A (dilution 1:10,000) and visualized using chemiluminescence.
The X-ray film was exposed for 30 seconds. Whereas a specific ~28 kDa band is detected in the GFP-tag control-lysate at all volumes (5 - 25 µl), no binding of the anti-GFP-tag antibody to cellular proteins can be detected in the lane, where 25 µl of the mock-lysate was applied.

Product

Whole cell lysate:
50 µl
Cell line:
HEK293 cells
Tagged protein:
Green fluorescent protein (GFP) from *Aequorea victoria*.
MW:
~28 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
20 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.

Use

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