tag-tools
www.tag-tools.de

tag-tools GmbH

Oberdorfstr. 16B
78465 Konstanz

mail@tag-tools.de
www.tag-tools.de

Immunoprecipitation

Materials

whole cell lysate (e.g. prepared using modified RIPA-buffer):

0.1% SDS
1% deoxycholic acid
1% Triton X-100
50 mM Hepes pH 7.4
150 mM NaCl
10% glycerol
1 mM EGTA
1.5 mM $MgCl_2$
10 mM sodium pyrophosphate
100 mM NaF
1 mM sodium orthovanadate
5 µg/ml leupeptin
10 µg/ml aprotinin
10 µg/ml pefabloc
5 µg/ml pepstatin
10 µM benzamidine

tag-tools tag-specific antibody (3 µg/sample)

protein A/G sepharose (25 µl/sample)

Procedure

This method allows enrichment of tagged proteins from complex protein mixtures such as whole cell lysats.

1. 3 µg of **tag-tools** tag-specific antibody is added to 500 µl of cleared whole cell lysate containing the tagged protein of interest.

   As a control, an irrelevant control antibody is added to a separate sample containing a similar lysate.
2. Samples are incubated on a rotating platform for 2-4 h at 4°C.
3. To precipitate the antigen-antibody complex, 25 µl of protein A/G-sepharose slurry is added to each sample and the samples are further rotated for 1h at 4°C.
4. Precipitates are collected by centrifugation (1 min, 2000g at 4°C), the supernatant is sucked off, and the sepharose beads are resuspended in 500 µl RIPA buffer.
5. The washing step (see 4.) is repeated 2 – 3 times and finally the precipitates are taken up in 2x SDS sample-buffer (total volume about 40 µl) for further analysis by SDS-PAGE and subsequent Western-blot.