Fluorescent immunoprecipitation analysis

Alexander V. Filatov, Grigory I. Krotov, Victor G. Zgoda, Yuri Volkov

Abstract

Surface protein labeling followed by immunoprecipitation

Citation: Alexander V. Filatov, Grigory I. Krotov, Victor G. Zgoda, Yuri Volkov Fluorescent immunoprecipitation analysis. **protocols.io**
dx.doi.org/10.17504/protocols.io.h5db826
Published: 30 Jun 2017

Protocol

Step 1.

Wash cells with cold PBS 2 times and resuspend in 1 ml of cold PBS.

Protein labeling

Step 2.

Add 30 µL of cyanine succinimidyl ester stock solution in dimethyl sulfoxide (10 mg/mL) to cells and incubate for 20 min on ice.

DURATION
00:20:00

Step 3.

Remove unreacted dye by washing cells with cold PBS 2 times.

Cell lysis

Step 4.

Lyse cells in lysis buffer (1 ml lysis buffer per 50 million cells) containing 20 mM Tris-HCl (pH 8.0), 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, and a mixture of inhibitors including 1 mM PMSF, 10 mM NaF, and 1 mM Na3VO4. Incubate for 30 min at 4 °C.

DURATION
00:30:00

Step 5.

Pellet cell debris by centrifugation at 20,000 g for 15 min at 4 °C.

DURATION
00:15:00

Sorbent preparation

Step 6.

Transfer affinity beads into tubes and wash 2 times with lysis buffer. The amount of beads neeaded depends on the antibody affinity and antibody/beads ratio as well as on cell lysate volume.

Wash beads by centrifugation at 3000 g for 30 s.

Lysate preclearance

Step 7.

Preclear cell lysates by overnight rotation at 4 °C with normal mouse IgG covalently linked to CNBr-Sepharose.

DURATION
18:00:00

Immunoprecipitation

Step 8.

Transfer precleared lysates to AffiGel Hz hydrazide agarose beads coupled to mAb.
Rotate samples for 2 h at 4 °C.

DURATION
02:00:00

Step 9.

Wash the beads 4 times with lysis buffer.

Protein elution

Step 10.

Elute proteins by heating the beads in SDS-sample buffer for 5 min at 80 °C.

DURATION
00:05:00

Protein elution

Step 11.

Centrifuge samples and collect eluates.