Cat # A0503 Store at –20 °C

Phospho-Akt (Ser 473) Antibody

200 µl
(50 Western mini-blots)
600 µl
(150 Western mini-blots)

SignaGen® Laboratories

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Applications	Species Cross-Reactivity	Molecular Wt.	Source
W, IP, F	H, M, R, Hm, C	60 kDa	Rabbit

Western Immunoblotting Protocol

Solutions and Reagents
Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X):
62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10%
glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer:
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for
150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g
nonfat dry milk and mix well. While stirring, add 0.15 ml
Tween-20 (100%).
10X TBS (Tris-buffered saline):
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl;
adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer:
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for
20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g
nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
Wash Buffer TBS/T:
1X TBS, 0.1% Tween-20
Blotting Membrane:
This protocol has been optimized for nitrocellulose membranes,
which we recommend. PVDF membranes may also be used.

Protein Blotting:
A general protocol for sample preparation using $2x10^6$ COS-7 cells per well in a 6-well plate is described below.
1. Culture cells in medium containing FBS for 2 days. We
recommends plating cells directly in 0.5% FBS media to reduce basal levels of phosphorylation.
2. Aspirate media. Add fresh 0.5% FBS media. Culture for 2 hours.
6. Sonicate for 10–15 seconds to shear DNA and reduce
sample viscosity.
7. Heat a 20 µl sample to 95~100°C for 5 minutes; cool on ice.
8. Microcentrifuge for 5 minutes.
9. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
10. Electrotransfer to nitrocellulose membrane.
Membrane Blocking and Antibody Incubations:
1. Incubate membrane in 25 ml of Blocking Buffer for 1 hour at
room temperature.
2. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
3. Incubate membrane and primary antibody (at the appropriate
dilution) in 10 ml Primary Antibody Dilution Buffer with gentle
agitation overnight at 4°C.
4. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
5. Incubate membrane with HRP-conjugated secondary antibody in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
6. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
Detection of Proteins
1. Incubate membrane with 10 ml ECL Reagent
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.
Note: Due to the kinetics of the detection reaction, signal is most intense immediately following ECL reagent incubation and declines over the following 2 hours.

Applications Key: W—Western / IP—Immunoprecipitation / IHC—Immunohistochemistry / IC—Immunocytochemistry / F—Flow cytometry / E—ELISA

Species Cross-Reactivity Key: H—human / M—mouse / R—rat / Hm—hamster / Mk—monkey / Mi—mink / C—chicken / X—*Xenopus* / Z—zebra fish / All—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.