#4766 Store at -20°C

# NF-κB Family Member Antibody Sampler Kit

1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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**For Research Use Only. Not For Use In Diagnostic Procedures.**

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|---|---|---|---|---|
| NF-κB p65 (L8F6) Mouse mAb | 6956 | 20 µl | 65 kDa | Mouse IgG2b |
| NF-κB p65 (D14E12) XP® Rabbit mAb | 8242 | 20 µl | 65 kDa | Rabbit IgG |
| RelB (C1E4) Rabbit mAb | 4922 | 20 µl | 70 kDa | Rabbit IgG |
| c-Rel (D4Y6M) Rabbit mAb | 12707 | 20 µl | 68-78 kDa | Rabbit IgG |
| NF-κB1 p105/p50 (D7H5M) Rabbit mAb | 12540 | 20 µl | 50 kDa Active form<br>120 kDa Precursor. | Rabbit IgG |
| NF-κB1 p105 Antibody | 4717 | 20 µl | 120 kDa | Rabbit IgG |
| NF-κB2 p100/p52 Antibody | 4882 | 20 µl | 52 kDa Active form<br>120 kDa Precursor | Rabbit IgG |
| NF-κB2 p100/p52 (18D10) Rabbit mAb (Human Specific) | 3017 | 20 µl | 52 kDa Active form<br>120 kDa Precursor | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |
| Anti-mouse IgG, HRP-linked Antibody | 7076 | 100 µl | | Horse |

**See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.**

**Description:** This kit contains reagents to examine total protein levels of the five NF-κB/Rel family members: p65/RelA, RelB, c-Rel, NF-κB1 (p105/p50) and NF-κB2 (p100/p52).

**Background:** Transcription factors of the nuclear factor κ B (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (1,2). There are five family members in mammals: RelA, c-Rel, RelB, NF-κB1 (p105/p50), and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. Rel proteins bind p50 and p52 to form dimeric complexes that bind DNA and regulate transcription. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins (3-5). NF-κB-activating agents can induce the phosphorylation of IκB proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF-κB to enter the nucleus where it regulates gene expression (6-8). NIK and IKKα (IKK1) regulate the phosphorylation and processing of NF-κB2 (p100) to produce p52, which is then translocated to the nucleus (9-11).

**Specificity/Sensitivity:** Each antibody in this kit recognizes endogenous levels of its target protein regardless of post-translational modification state such as phosphorylation or acetylation. The NF-κB1 p105/p50 (D7H5M) Rabbit mAb #12540 detects both the precursor protein p105 and its cleavage product p50, while the NF-κB1 p105 Antibody #4717 only detects p105 and will not cross-react with p50. Both the NF-κB2 p100/p52 Antibody #4882 and the NF-κB2 p100/p52 (18D10) Rabbit mAb (Human Specific) #3017 will cross-react with the precursor protein p100 and its cleavage product p52.

**Source/Purification:** Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to amino acid residues near the carboxy terminus of human NF-κB p65, surrounding Ser424 of human RelB, surrounding Leu65 of human c-Rel protein, surrounding Gly415 of human NF-κB p105/p50 protein, surrounding Glu498 of human NF-κB p65/RelA protein, and near the amino terminus of human NF-κB2 p100/p52. Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to amino acid residues at the the carboxy terminus of human NF-κB1 p105 and near the amino terminus of human NF-κB2 p100/p52. Polyclonal antibodies are purified by Protein A and peptide affinity chromatography.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

**Recommended Antibody Dilutions:**

| | |
|---|---|
| Western blotting | 1:1000 |

**Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.**

**Background References:**

(1) Baeuerle, P.A. and Henkel, T. (1994) *Annu Rev Immunol* 12, 141–79.
(2) Baeuerle, P.A. and Baltimore, D. (1996) *Cell* 87, 13–20.
(3) Haskill, S. et al. (1991) *Cell* 65, 1281–9.
(4) Thompson, J.E. et al. (1995) *Cell* 80, 573–82.
(5) Whiteside, S.T. et al. (1997) *EMBO J* 16, 1413–26.
(6) Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876–83.
(7) Scherer, D.C. et al. (1995) *Proc Natl Acad Sci USA* 92, 11259–63.
(8) Chen, Z.J. et al. (1996) *Cell* 84, 853–62.
(9) Senftleben, U. et al. (2001) *Science* 293, 1495–9.
(10) Coope, H.J. et al. (2002) *EMBO J* 21, 5375–85.
(11) Xiao, G. et al. (2001) *Mol Cell* 7, 401–9.

U.S. Patent No. 5,675,063



**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#4766

# Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
**NOTE:** Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

## A. Solutions and Reagents

**NOTE:** Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml $dH_2O$, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml $dH_2O$, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with $dH_2O$.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml $dH_2O$, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml $dH_2O$, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml $dH_2O$, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

## B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

## C. Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 $cm^2$) of membrane; for different sized membranes, adjust volumes accordingly.

### I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

### II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

## D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.