Technical Data Sheet

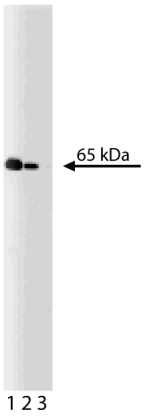
Purified Mouse Anti- NF-κB p65

Product Information

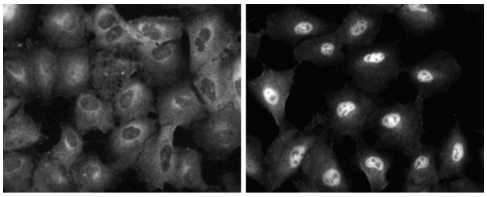
Material Number:	**610869**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	20/NF-kB/p65
Immunogen:	Human NF-κB aa. 136-224
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Dog, Frog, Rabbit, Rat
Target MW:	65 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

NF-κB is a ubiquitously expressed transcription factor that regulates many cytokine and Ig genes. It is involved in immune, inflammatory, viral, and acute phase responses. The most studied NF-κB complex consists of the p50 and p65 subunits, both containing a 300 amino acid region with homology to the *Rel* proto-oncogene product. The p50 subunit binds DNA, whereas the p65 subunit is responsible for the interaction of NF-κB with its inhibitor, IκB. In most cell types, the p50/p65 heterodimer is located within the cytoplasm complexed to IκB. This complex prevents nuclear translocation and activity of NF-κB. In response to stimuli such as cytokines, LPS, and viral infections, IκB is phosphorylated at critical residues. This phosphorylation induces dissociation of the IκB/NF-κB complex, allowing the free heterodimeric NF-κB to form a heterotetramer that translocates to the nucleus. In the nucleus, it binds to the κB site within promoters and enhancers and functions as a transcriptional activator.



Western blot analysis of NF-κB p65 on a Jurkat lysate. *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of NF-κB p65 antibody.*



Immunofluorescent staining of A549 (ATCC CCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were either mock treated (media, left) or exposed to TNF (20ng/ml, right) for 15 minutes. After treatment cells were stained using the Triton™ X-100 perm protocol and the anti-NF-κB antibody. The second step reagent was Alexa-Fluor® 488 goat anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager with a 20x objective. This antibody also stains U-2 OS (ATCC HTB-96) and HeLa (ATCC CCL-2) cells and can be used with either perm protocol (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
611451	Jurkat Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Baeuerle PA, Baltimore D. Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-kappa B transcription factor. *Cell.* 1988; 53(2):211-217. (Biology)

Kieran M, Blank V, Logeat F, et al. The DNA binding subunit of NF-kappa B is identical to factor KBF1 and homologous to the rel oncogene product. *Cell.* 1990; 62(5):1007-1018. (Biology)

Nishibe T, Parry G, Ishida A, et al. Oncostatin M promotes biphasic tissue factor expression in smooth muscle cells: evidence for Erk-1/2 activation. *Blood.* 2001; 97(3):692-699. (Clone-specific: Western blot)

Pimentel-Muinos FX, Seed B. Regulated commitment of TNF receptor signaling: a molecular switch for death or activation. *Immunity.* 1999; 11(6):783-793. (Clone-specific: Western blot)

Shirakawa F, Mizel SB. In vitro activation and nuclear translocation of NF-kappa B catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. *Mol Cell Biol.* 1989; 9(6):2424-2430. (Biology)