Purified anti-NF-κB p65 Antibody

Catalog# / Size	622601 / 50 µl (5 Western blots) 622602 / 200 µl (20 Western blots)
Clone	Poly6226
Regulatory Status	RUO
Other Names	NFκB p65, nuclear factor kappa light chain enhancer in B cells p65
Isotype	Rabbit Polyclonal IgG
Description	NF-κB/p65 (nuclear factor kappa light chain enhancer in B cells p65) is a member of the Rel/dorsal family. This ubiquitously expressed nuclear protein is one subunit of the NF-kappa B complex consisting of a 65 kD transactivating subunit and a 50 kD DNA binding subunit. Both subunits are derived from larger precursor proteins. NF-κB acts as a transcriptional activator and was originally identified as an activator of kappa light chain in B cells. NF-κB/p65 is bound (with p50) to IκB inhibitor in cytoplasm in inactive form. Phosphorylation of IκB releases and NF-κB which goes to the nucleus to activate gene expression. NF-κB can be activated with LPS, TNF-α, phorbol ester, or IL-1. The Poly6226 antibody reacts with the C-terminal region of human, mouse, and rat NF-κB p65 and has been shown to be useful for Western blotting.

Product Details

Verified Reactivity	Mouse, Rat, Human
Antibody Type	Polyclonal
Host Species	Rabbit
Immunogen	peptide-KLH, C-terminal
Formulation	This antibody is provided in phosphate-buffered solution, pH 7.2, containing 0.1% sodium azide and 0.2% gelatin.
Preparation	The antibody was purified by antigen-affinity chromatography.
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	Upon receipt, store undiluted between 2°C and 8°C.
Application	WB - Quality tested ICC, ChIP - Verified IHC-F, IP - Reported in the literature, not verified in house
Recommended Usage	Each lot of this antibody is quality control tested by Western blotting. For Western blotting, the suggested use of this reagent is 0.05 µg per mL. For immunocytochemistry, the suggested use of the reagent is a dilution range of 1:100~1:400. For ChIP applications, the suggested dilution is 1:50-1:200 by volume. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	This product may contain other non-IgG subtypes.
Application References **(PubMed link indicates BioLegend citation)**	1. Chen R, *et al.* 2014. *J Mol Cell Cardiol.* 72:263. PubMed 2. Tan GK, *et al.* 2014. *Acta Biomater.* 10:2684. PubMed 3. Abecassis A, *et al.* 2014. *Cell Mol Immunol.* 4:377. PubMed 4. Sumedha N, *et al.* 2014. *Hum Exp Toxicol.* 34:506. PubMed 5. Hua X, *et al.* 2015. *PloS One.* 10:128039. PubMed
Product Citations	1. Fang SY, *et al.* 2021. Exp Ther Med. 369:21. PubMed 2. Smaldone G, *et al.* 2021. Sci Rep. 11:18237. PubMed 3. Haddad H, *et al.* 2021. Int J Mol Sci. 22:. PubMed 4. Hua X, *et al.* 2015. PLoS One. 10:128039. PubMed 5. Abecassis A, *et al.* 2014. Cell Mol Immunol. 4:377. PubMed

6. Chung H, *et al.* 2021. Nat Methods. 18:1204. PubMed
7. Afzal SM, *et al.* 2020. Environ Toxicol. :. PubMed
8. Chaudhari S, *et al.* 2020. Am J Physiol Renal Physiol. 318:F1478. PubMed
9. Ayari C, *et al.* 2011. J Urol. 185:1915. PubMed
10. Ge Y, *et al.* 2013. J Virol. 87:11160. PubMed
11. Miltonprabu N 2014. Hum Exp Toxicol. 34:506. PubMed
12. Yang Z, *et al.* 2016. Sci Rep. 6:30263. PubMed
13. Muri J, *et al.* 2020. eLife. 9:e53627.. PubMed
14. Tortola L, *et al.* 2022. Mucosal Immunol. 15:656. PubMed
15. Tan G, Tabata Y 2014. Acta Biomater. 10:2684. PubMed
16. Chen R, *et al.* 2014. J Mol Cell Cardiol. 72:263. PubMed

RRID

AB_315955 (BioLegend Cat. No. 622601)
AB_315956 (BioLegend Cat. No. 622602)

Antigen Details

Structure

Member of the Rel/dorsal family. One subunit of the NF-kappa B complex consisting of a 65 kD transactivating subunit and a 50 kD DNA binding subunit. Both subunits derived from larger precursor proteins

Distribution

Ubiquitously expressed nuclear protein

Function

Transcriptional activator, originally identified as an activator of kappa light chain in B cells

Interaction

Interacts with NF-κB p50 and IκB

Biology Area

Cell Biology, Neuroscience, Neuroscience Cell Markers, Transcription Factors

Molecular Family

Nuclear Markers

Antigen References

1. Baldwin AS, *et al.* 1996. *Annu. Rev. Immunol.* 14:649 (review).
2. Chen F, *et al.* 1999. *Clin. Chem.* 45:7 (review).

Regulation

Bound (with p50) to IκB inhibitor in cytoplasm in inactive form. Phosphorylation of IκB releases and NF-κB goes to the nucleus to activate gene expression. Stimulation with LPS, TNF-α, phorbol ester, or IL-1 activates

Gene ID

4790

Related Protocols

BioLegend's Tools for Chromatin Immunoprecipitation (ChIP) Assays - Video

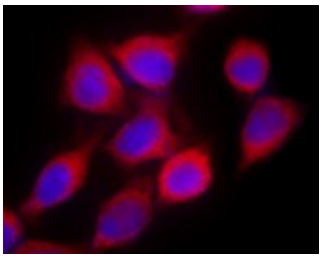
Chromatin Immunoprecipitation (ChIP) Assay Protocol

Western Blotting Protocol

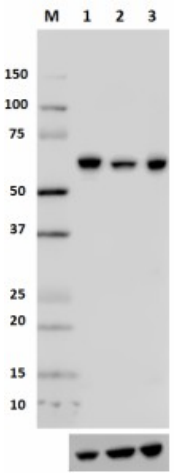
Other Formats

Purified anti-NF-κB p65

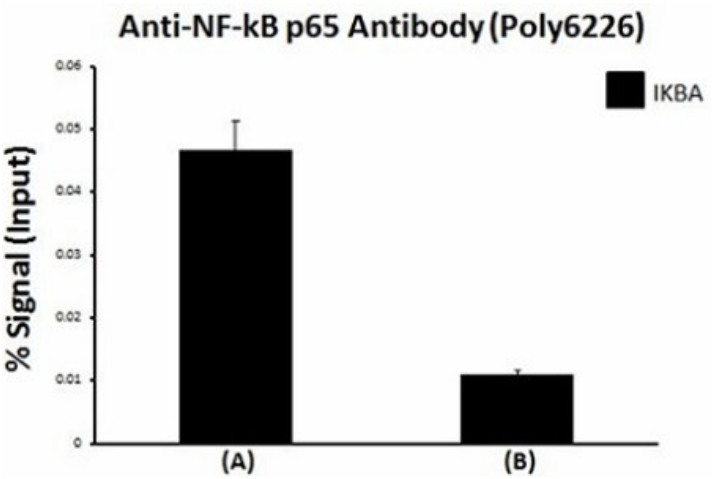
Product Data



Hela cells stained with purified rabbit polyclonal antibody against NF-κB p65 (poly6226), followed by Rhodamine Red-X conjugated goat anti-rabbit IgG and DAPI



Total lysates (15 µg protein) from HeLa (lane 1), Raw 264.7 (lane 2) and UMR106 cells (lane 3) were resolved by electrophoresis (4-20% Tris-Glycine gel), transferred to nitrocellulose, and probed with 0.05 µg/mL Purified anti-NF-κB p65 Antibody, clone poly6226 (upper). Proteins were visualized by chemiluminescence detection using a 1:3000 diluted goat anti-rabbit-IgG secondary antibody conjugated to HRP for the anti-NF-κB p65 Antibody. 1:5000 diluted Direct-Blot HRP anti-β-Actin Antibody (2F1-1) was used as a loading control (lower). Lane M: Molecular weight ladder.



Chromatin Immunoprecipitation (ChIP) was performed using Go-ChIP-Grade™ protein G Enzymatic kit by loading 3 µg of cross-linked chromatin samples from HeLa cells treated with TNFα with either A) 1:100 dilution of Go-ChIP-Grade™ Purified anti-NF-κB p65 (Clone Poly6226), or B) equal amount of Purified Rabbit Polyclonal Isotype Control Antibody (Cat. No. 910801). The enriched DNA was purified and quantified by real-time qPCR using primers targeting human IKBA gene region. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the 5% of total amount of input chromatin.

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587