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Datasheet for 100-401-266

NFkB p65 (RelA) Phospho S529 Antibody

Overview

Description:	Anti-NFKB p65 (Rel A) pS529 (RABBIT) Antibody - 100-401-266
Item No.:	100-401-266
Size:	100 µL
Applications:	ELISA, WB, IF
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	NFkB was originally identified as a factor that binds to the immunoglobulin kappa light chain enhancer in B cells. It was subsequently found in non-B cells in an inactive cytoplasmic form consisting of NFkB bound to IkB. NFkB was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NFKB1) subunits. Other identified subunits include p52 (NFKB2), cRel, and RelB. The p65, cRel, and RelB subunits are responsible for transactivation. The p50 and p52 subunits possess DNA binding activity but limited ability to transactivate. p52 has been reported to form transcriptionally active heterodimers with the NFkB subunit p65, similar to p50/p65 heterodimers. Low levels of p52 and p50 homodimers can also exist in cells. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IkB-alpha. IkB-alpha binds to the p65 subunit preventing nuclear localization and DNA binding. Activators mediate a rapid phosphorylation of IkB by IkB kinase (IKK) which results in subsequent ubiquitination and proteolytic degradation. NFkB is then transported to the nucleus, where it activates transcription of target genes through binding to NFkB target sequences within the promoter. The HTLV-I protein Tax can induce constitutive NFkB activation through phosphorylation of both IkB-alpha and IkB-beta. The transforming protein Tax inhibits p53 transcriptional activity through the NFkB signaling pathway, specifically via the p65 (RelA) subunit. Anti-NFkB antibody is ideal for Cell Biology, Nuclear Signaling, Neuroscience and Signal Transduction Research.
Synonyms:	rabbit anti-NFkB p65 pS529 Antibody, rabbit anti-RelA pS529 Antibody, NFKB, nfkb, NF-kB, NF-kappaB, NFkappaB
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	RELA
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	NFkB p65 (Rel A) peptide corresponding to a region near phospho Serine 529 of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	Anti-NFkB p65 (RelA) Phospho S529 Antibody was prepared from monospecific antiserum by delipidation and defibrination. This phospho specific polyclonal antibody is specific for phosphorylated pS529 human p65. Reactivity with non-phosphorylated p65 is minimal. Cross reactivity with pS529 phosphorylated p65 from mouse, rat or other species has not been determined.
Relevant Links:	• UniProtKB - Q04206 • NCBI - 223468676 • GeneID - 5970

Application Details

Tested Applications:	ELISA, WB
Suggested Applications:	IF (Based on references)
Application Note:	Anti-phospho NFkB antibody reacts human pS529 p65 and shows minimal reactivity by western blot with non-phosphorylated p65 and minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. Although not tested, this antibody is likely functional in immunohistochemistry and immunoprecipitation.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:25,000
IHC:	1:200 - 1:1,000
WB:	1:500 - 1:3,000

Formulation

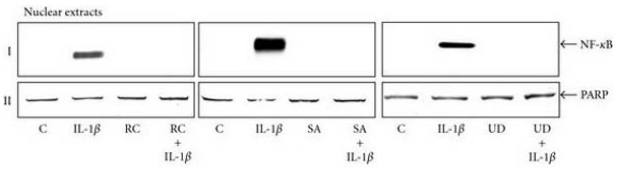
Physical State:	Liquid (sterile filtered)

Concentration:	75 mg/mL by Refractometry
Buffer:	None
Preservative:	0.1% (w/v) Sodium Azide
Stabilizer:	None

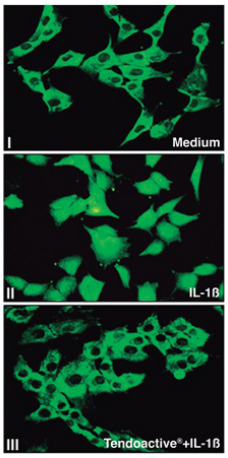
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store NFkB p65 (RelA) Phospho S529 Antibody at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

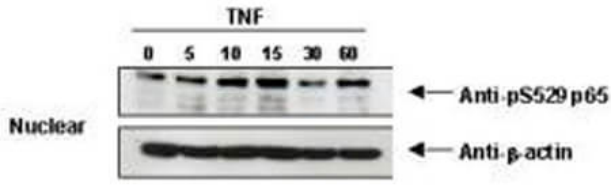
Images



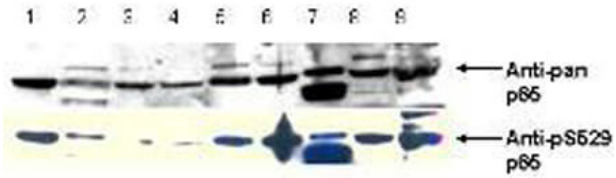
Western Blot
Botanical extracts block the IL-1Œ≤-induced phosphorylation and nuclear translocation of p65 in chondrocytes. Western blot analysis of IL-1Œ≤-treated nuclear extracts. Serum-starved chondrocytes (0.1 √ó 106 cells/mL) were pretreated with botanical extracts (10,ÄâŒºg/ML each) for 4 hours followed by cotreatment with 10,Äâng/mL IL-1Œ≤ and botanical extracts for 1,Äâh. Some chondrocyte cultures remained either untreated or were treated with 10 ,ÄâŒºg/mL botanical extracts (each alone) or with 10 ,Äâng/mL IL-1Œ≤ alone for 1,Äâh. Nuclear extracts were probed for phospho p65, (I) by western blot analysis using antibodies to p65, phospho-specific p65, and PARP (II, control). Treatment of chondrocytes with IL-1Œ≤ (10 ,Äâng/mL) revealed a clear increase in expression of phospho-p65 in the nuclear extracts (I). Co-treatment of chondrocytes with botanical extracts (all three) completely abolished the IL-1Œ≤-dependent activation of phospho p65 in the nucleus (I). Synthesis of PARP remained unaffected in nuclear extracts (II). Data shown are representative of three independent experiments. Treatments: C (untreated control); IL-1Œ≤; RC (Rosa canina); SA (Salix alba); UD (Urtica dioica). Figure provided by CiteAb. Source: Evid Based Complement Alternat Med, PMID: 22474508.



Immunofluorescence Microscopy
Tendoactive® inhibited IL-1ß-induced nuclear translocation of phospho p65 revealed by immunofluorescence microscopy. Tenocytes cultures either served as controls (I, not treated) or were treated with 10 ng/ml IL-1ß alone for 10 min or pre-treated first with Tendoactive® (250 µg/ml) for 1h alone and then were co-treated with 10 ng/ml IL-1ß for 10 min before indirect immunolabeling with anti-phospho p65 antibody and FITC-coupled secondary antibodies. In control cells anti-phospho p65 labelling was restricted to the cytoplasm (I). Cells treated with IL-1ß alone revealed nuclear translocation of phospho p65 (II) that was partly inhibited by co-treatment with Tendoactive® (III). Data shown are representative of three independent experiments. X160. Fig 5. PMID: 21751149.



Western Blot
TNF Induces phosphorylation of p65 in KBM-5 cells. Nuclear protein lysates prepared after 0, 5, 10, 15, 30 and 60 minutes of 0.1 nM TNF treatment of KBM-5 cells shows inducible phosphorylation using phospho specific polyclonal anti-human pS529 p65. Anti-beta-actin staining confirms loading of equivalent amounts of protein. HRP conjugated Gt-anti-Rabbit IgG was used to develop the blot using a chemiluminescent detection method. Other detection methods will yield similar results. Data contributed by Aggarwal BB, personal communication.



Western Blot
Anti-pS529 shows phospho p65 staining in carcinoma cells. western blot of total protein lysates from various human head and neck tumors shows phospho p65 staining in tumor cell lines using phospho specific polyclonal anti-human pS529 p65. Lanes 1-6 contain protein lysates from human squamal carcinoma cell lines. Lane 7 is a protein lysate from a primary culture of human keratinocytes. Lane 8 contains protein lysate from ATCC SCC9 cells (also a head and neck squamal carcinoma). Lane 9 contains lysate from EGF-induced human derived A431 cells. Lane 10 (not shown) contains a molecular weight standard. Concurrent staining with anti-beta microtubulin (not shown) was used to confirm equal protein loading in all lanes. HRP conjugated Gt-anti-Rabbit IgG was used to develop the blot using a chemiluminescent detection method. Other detection methods will yield similar results. Data contributed by Yu, M., NIH, personal communication.

References

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