diagenode
Innovating Epigenetics Solutions

ChIP-seq grade

TECHNICAL DATASHEET

NFKB p65 polyclonal antibody

Cat. No. C15310256

Type: Polyclonal	Specificity: Human, mouse, rat
Size: 100 µl	Isotype: NA
Concentration: not determined	Host: Rabbit
Lot No.: 24468	Purity: Whole antiserum
Storage buffer: NA	Storage conditions: NA
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.	

Description

Alternative names: **RELA**, **NFKB3**, **p65**

Polyclonal antibody raised in rabbit against NFKB p65 (Rel A), using a KLH-conjugated synthetic peptide.

Applications

Applications	Suggested dilution	References
ChIP *	1 µl/IP	Fig 1, 2
Western Blotting	1:2,000 - 1:5,000	Fig 3
ELISA	1:5,000	
Immunohistochemistry	1:500 - 1:2,000	Fig 4
Gel Shift	1:500	

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

Target Description

This antibody recognizes NFKB p65 which is a component of NFKB. 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 NFkappaB bound to IkappaB. NFkappaB was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NFKB1) subunits. Other identified subunits include p52 (NFKB2), c-Rel, 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 NFkappaB subunit p65, similar to p50/p65 heterodimers. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IkB-a. IkB-a binds to the p65 subunit, preventing nuclear localization and DNA binding. Low levels of p52 and p50 homodimers can also exist in cells.

Diagenode sa. BELIGUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing (Ougrée) - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
info@diagenode.com | orders@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
info.na@diagenode.com | orders.na@diagenode.com

Validation Data

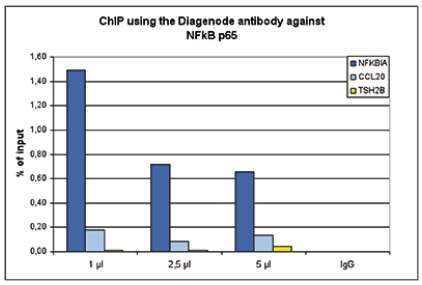

Figure 1. ChIP results obtained with the Diagenode antibody directed against NFkB p65.

ChIP assays were performed using human HeLa cells, treated with TNFalpha, the Diagenode antibody against NFkB p65 (Cat. No. C15310256) and optimized PCR primer sets for qPCR. ChIP was performed with the "iDeal ChIP-seq" kit (Cat. No. C01010055), using sheared chromatin from 4 million cells. A titration of the antibody consisting of 1, 2.5 and 5 µl per ChIP experiment was analysed. IgG (1 µg/IP) was used as negative IP control. QPCR was performed with primers for the NFKBIA and CCL20 genes, used as positive controls, and for TSH2B, used as negative control. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

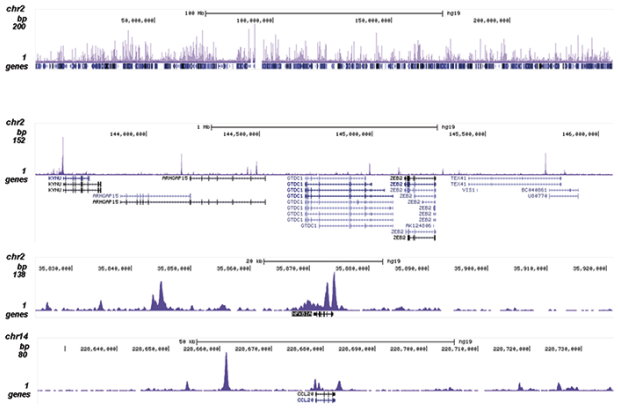

Figure 2. ChIP-seq results obtained with the Diagenode antibody directed against NFkB p65

ChIP was performed on sheared chromatin from 4 million HeLa cells using 1 µg of the Diagenode antibody against NFkB p65 (Cat. No. C15310256) as described above. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure 2 shows the enrichment along the complete sequence and a 2 Mb region of human chromosome 2 (fig 2A and B), and in a two genomic regions surrounding the NFKBIA and CCL20 positive control genes.

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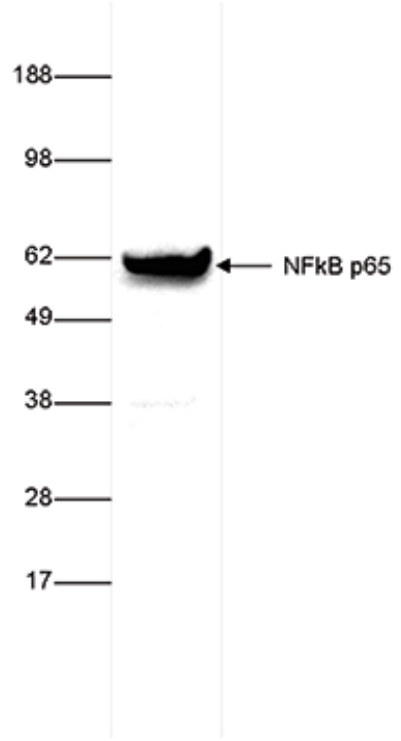

Figure 3. NFKB p65 antibody western blot results
Whole cell extracts from HeLa cells (35 µg) were analysed by Western blot using the Diagenode antibody against NFkB p65 (Cat. No. C15310256) diluted 1:5,000. The position of the protein of interest is indicated on the right (expected size: 65 kDa); the marker (in kDa) is shown on the left.

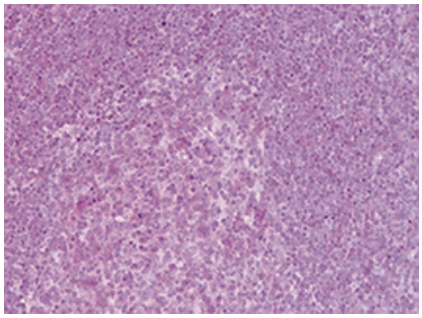

Figure 4. NFKB p65 antibody Immunohistochemistry results Formalin fixed paraffin embedded lymphocytes and germinal center cells of the tonsil were stained with the Diagenode antibody against NFkB p65 (Cat. No. C15310256) diluted 1:400 followed by a peroxidase labelled goat anti-rabbit secondary antibody. Figure 4 shows moderate positive nuclear or cytoplasmic staining.

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