

### Product Specification Sheet

**Product:** anti-NFκB p50 (NFKB1) [Rabbit]

**Code:** 100-4164

**Lot #** 14593

**Size:** 100 μl

**Physical State:** Liquid (sterile filtered)

**Protein Concentration:** 80.0 mg/ml (by Refractometry)

**Buffer:** None

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Application(s):** Suitable for immunoprecipitation, immunoblotting, ELISA and supershift assays.

**Background:** NFκB 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 NFκB bound to IκB. NFκB 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 NFκB subunit p65, similar to p50/p65 heterodimers. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IκB-α. IκB-α binds to the p65 subunit, preventing nuclear localization and DNA binding. Low levels of p52 and p50 homodimers can also exist in cells.

**Gel (Super) Shift Information:** In general, NFκB gel shift assays are assembled in 20μl reactions containing 0.28 pmoles NFκB oligo in 10mM Tris (pH 7.6), 50 mM NaCl, 0.5 mM EDTA, 1.0 mM DTT, 10% glycerol. Some procedures specify the addition of 0.05% NP-40. When using purified protein, 250-300 ng should be sufficient to produce a gel shifted complex, while 10μg HeLa nuclear extract is utilized. The gel shift reactions are then incubated at room temperature for 30 minutes. The complexes are resolved on a Tris-Glycine acrylamide gels. Loading dye containing bromophenol blue and xylene cyanol should be added to the negative control reaction **only**, as these dyes can increase the dissociation of the NFκB complexes. When using HeLa nuclear extract as the source of binding proteins, two sequence-specific gel-shifted complexes are expected, consisting of p50/p50 homodimers and p50/p65 heterodimers. For cells expressing p52, p50, and p65, as many as four sequence-specific gel-shifted complexes could be observed (p52/p52, p50/p50, p52/p65, p50/p65), and if high levels of p65 are present, the p65/p65 homodimer may also be weakly detected. The following reagents have been observed to enhance NFκB binding *in vitro*: millimolar amounts of GTP and ATP, spermine, spermidine, barium or calcium ions, and μM amounts of Co<sup>+3</sup>(NH<sub>3</sub>)<sub>6</sub>.

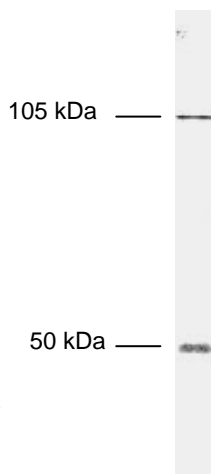
**Recommended Dilution(s):** This product was assayed by immunoblot and found to be reactive against Human NFκB p50 (NFKB1) at a dilution of 1:1000 followed by reaction with Peroxidase conjugated Affinity Purified anti-Rabbit IgG [H&L] (Goat) code #611-1302. Anti-Human NFκB p50 (NFKB1) is suitable for the detection by immunoblot of Human NFκB p50 (NFKB1) and its precursor protein p105. No reaction was observed against the analogous mouse protein. This product was also tested in a gel supershift assay and found to be reactive against p50:p50 homodimers and p:50:p65 heterodimers using 0.5 to 1.0 μl per assay. Optimal titers for other applications should be determined by the researcher.

**Storage Conditions:** Store vial at -20° C or below prior to opening. Dilute only prior to immediate use. Aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is six (6) months from date of opening product.

**Purity:** This product was prepared from monospecific antiserum by delipidation and defibrination. Anti-Human NFκB p50 (NFKB1) may react non-specifically with other proteins. Control peptide (code #100-4164p) will compete only with the specific reaction of antiserum with Human NFκB p50 (NFKB1).

**Immunogen:** Human NFκB p50 (NFKB1) peptide corresponding to a region near the N-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.



**Figure.** Immunoblot of HeLa cell extract. All incubations except color development were performed using TBS supplemented with 0.1% Tween-20 at room temperature. The membrane was blocked in 5% dry milk for 2 h. After washing, a 1:1,000 dilution of the primary antibody was added to the membrane and incubated for 2 h. Washes with buffer were performed 4 times for 5' each. The immunoblot was incubated with secondary antibody (HRP Goat-a-Rabbit IgG [H&L]) diluted 1:2,000 for 1 h. Washes with TBS preceded color development.