

Product Specification Sheet

Product: anti-Human NF κ B p52 (NFKB2) [Rabbit]

Code: 100-4185

Lot # 3756

Size: 100 μ l

Physical State: Liquid (sterile filtered)

Antibody Concentration: 90.0 mg/ml (by Refractometry)

Buffer: None

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

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

Background: NF κ B 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 NF κ B bound to I κ B. NF κ B 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 NF κ B subunit p65, similar to p50/p65 heterodimers. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by an inhibitor called I κ B- α . I κ B- 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, NF κ B gel shift assays are assembled in 20 μ l reactions containing 0.28 pmoles NF κ B 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 10 μ g 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 NF κ B 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 NF κ B binding *in vitro*: millimolar amounts of GTP and ATP, spermine, spermidine, barium or calcium ions, and μ M amounts of Co⁺³(NH₃)₆.

Recommended Dilution(s): This product was assayed by immunoblot and found to be reactive against Human NFKB2 p52 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 NFKB2 p52 is suitable for the detection by immunoblot of Human NFKB2 p52 and its precursor protein p100. Cross reactivity with p52 from other species may occur but has not been specifically determined. Reactivity in supershift assays has not been determined. 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 NFKB2 p52 may react non-specifically with other proteins. Control peptide (code #100-4185p) will compete only with the specific reaction of antiserum with Human NFKB2 p52.

Immunogen: Human NFKB2 p52/p100 peptide corresponding to aa residue 1-19 the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).

Peptide Sequence: M-E-S-C-Y-N-P-G-L-D-G-I-I-E-Y-D-D-F-K

General Reference: Wang, Y., et al. (2002) NFKB2 p100 is a pro-apoptotic protein with anti-oncogenic function. *Nature Cell Biol.* 4:11;888-893.

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.