

Product datasheet

Anti-NF-kB p65 antibody ab16502

KO VALIDATED

★★★★☆ 40 Abreviews 1055 References 13 Images

Overview

Product name	Anti-NF-kB p65 antibody
Description	Rabbit polyclonal to NF-kB p65
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, IHC-P, WB, IP
Species reactivity	Reacts with: Mouse, Human Predicted to work with: Rat, Chicken, Indian muntjac, Heterocephalus glaber 
Immunogen	Synthetic peptide corresponding to Human NF-kB p65 aa 500 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as ab16636)
General notes	<p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Protein A purified

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab16502 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (9)	Use a concentration of 1 - 5 µg/ml.
IHC-P	★★★★★ (4)	Use a concentration of 1 - 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★★ (14)	Use a concentration of 0.5 µg/ml. Detects a band of approximately 64 kDa (predicted molecular weight: 60 kDa). Abcam recommends using milk as the blocking agent.
IP	★★★★★ (1)	Use at an assay dependent concentration.

Target

Function

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFkB1/p105, NFkB1/p50, REL and NFkB2/p52 and the heterodimeric p65-p50 complex appears to be most abundant one. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors, respectively. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I-kappa-B) family. In a conventional activation pathway, I-kappa-B is phosphorylated by I-kappa-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-kappa-B complex which translocates to the nucleus. NF-kappa-B heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-kappa-B p65-p65 complex appears to be involved in invasion-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B in the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1.

Sequence similarities

Contains 1 RHD (Rel-like) domain.

Domain

the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.

Post-translational modifications

Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response.

Monomethylated at Lys-310 by SETD6. Monomethylation at Lys-310 is recognized by the ANK repeats of EHMT1 and promotes the formation of repressed chromatin at target genes, leading to down-regulation of NF-kappa-B transcription factor activity. Phosphorylation at Ser-311 disrupts the interaction with EHMT1 without preventing monomethylation at Lys-310 and relieves the repression of target genes.

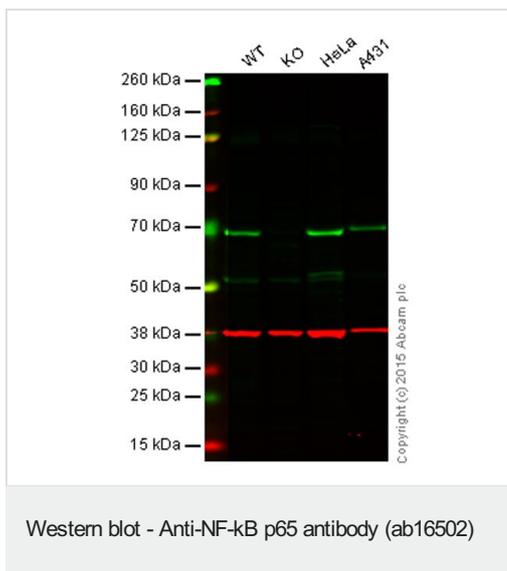
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

Reversibly acetylated; the acetylation seems to be mediated by CBP, the deacetylation by HDAC3. Acetylation at Lys-122 enhances DNA binding and impairs association with NFKBIA. Acetylation at Lys-310 is required for full transcriptional activity in the absence of effects on DNA binding and NFKBIA association. Acetylation can also lower DNA-binding and results in nuclear export. Interaction with BRMS1 promotes deacetylation of 'Lys-310'.

Cellular localization

Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha induction.

Images



All lanes : Anti-NF-kB p65 antibody (ab16502) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate

Lane 2 : NF-kB p65 knockout HAP1 cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

Predicted band size: 60 kDa

Lane 1 : Wild-type HAP1 cell lysate (20 µg)

Lane 2 : NFκB p65 knockout HAP1 cell lysate (20 µg)

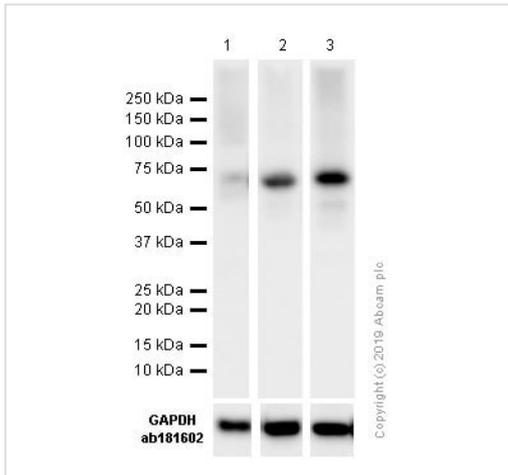
Lane 3 : HeLa cell lysate (20 µg)

Lane 4 : A431 cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab16502 observed at 70 kDa. Red - [ab8245](#) loading control, observed at 37 kDa.

ab16502 was shown to react with NFκB p65 in wild-type HAP1

cells along with additional cross-reactive bands. No band was observed when NFκB p65 knockout samples were used. Wild-type and NFκB p65 knockout samples were subjected to SDS-PAGE. ab16502 (NFκB p65) and **ab8245** (loading control to GAPDH) were both diluted 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-NF-κB p65 antibody (ab16502)

All lanes : Anti-NF-κB p65 antibody (ab16502) at 1/1000 dilution

Lane 1 : Human fetal brain lysates

Lane 2 : Human fetal kidney lysates

Lane 3 : Human fetal lung lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

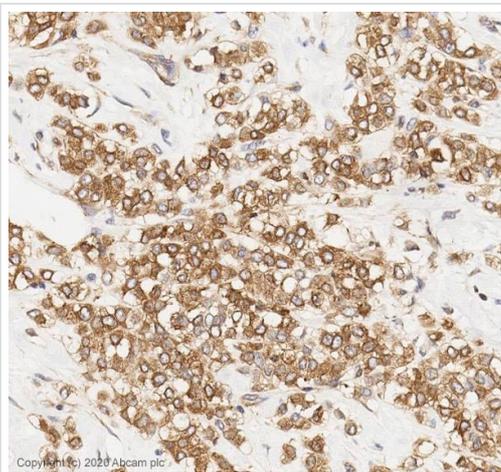
Predicted band size: 60 kDa

Observed band size: 65 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

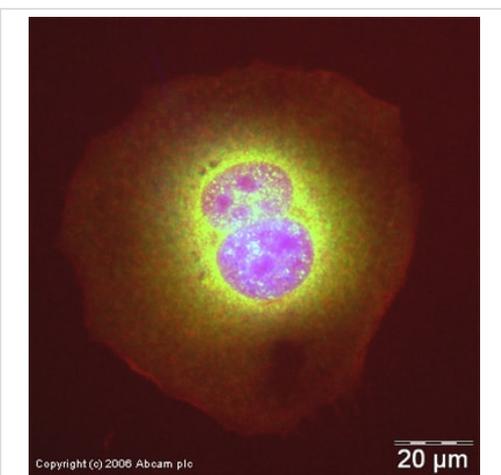
Exposure Time: 15 seconds for Lane1 and 3 seconds for Lanes 2 and 3

Normal brain might express low level of p65 (PMID: 21479220)



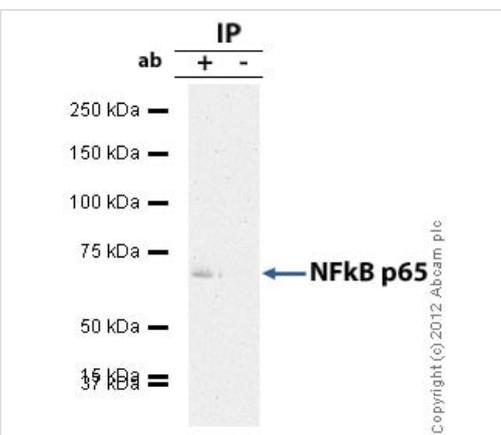
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody (ab16502)

IHC image of Anti-NF-kB p65 antibody staining in a section of formalin-fixed paraffin-embedded human breast adenocarcinoma performed on a Leica BOND™ system using the standard protocol. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab16502, 1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody (ab16502)

ICC/IF image of ab16502 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab16502, 1μg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 phalloidin was used to label F-actin (red).



Immunoprecipitation - Anti-NF-kB p65 antibody (ab16502)

NF-kB p65 was immunoprecipitated using 0.5mg Hela whole cell extract, 5μg of Rabbit polyclonal to NFkB p65 and 50μl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40μl SDS loading buffer and incubated for 10min at 70°C; 10μl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab16502.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody **Anti-Rabbit HRP** (IgG light chain) (**ab99697**).

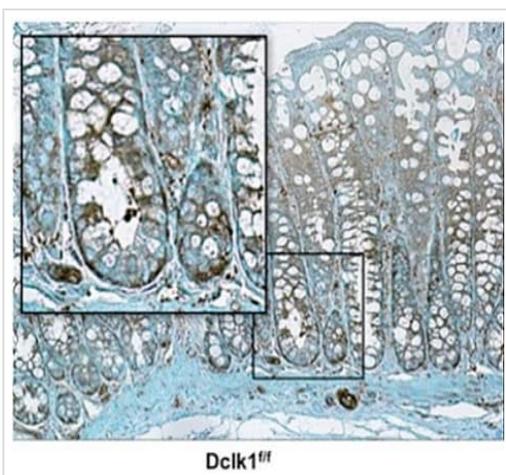
Band: 68kDa: NFkB p65



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody (ab16502)

Image from Qu D et al. PLoS ONE. 2015; 10(8): e0134212. Fig 5. doi:10.1371/journal.pone.0134212. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Villin^{Cre};Dclk1^{ff} mouse colon tissue sections labeling NF-kB p65 with ab16502 (brown). Alcian blue was used for counterstaining. Heat-induced epitope retrieval was performed on 4- μ m formalin-fixed paraffin-embedded sections by utilizing a pressurized Decloaking Chamber in citrate buffer (pH 6.0) at 99°C for 18 min. For brightfield microscopy, slides were exposed to peroxidase blocking solution prior to the addition of primary antibody (ab16502). After incubation with primary antibody overnight at 4°C, the slides were incubated in peroxidase-conjugated polymer.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody (ab16502)

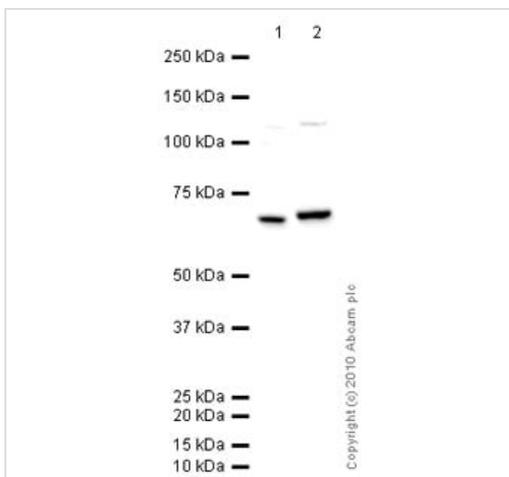
Image from Qu D et al. PLoS ONE. 2015; 10(8): e0134212. Fig 5. doi:10.1371/journal.pone.0134212.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Dclk1^{ff} mouse colon tissue sections labeling NF-kB p65 with ab16502 (brown). Alcian blue was used for counterstaining. Heat-induced epitope retrieval was performed on 4- μ m formalin-fixed paraffin-embedded sections by utilizing a pressurized Decloaking Chamber in citrate buffer (pH 6.0) at 99°C for 18 min. For brightfield microscopy, slides were exposed to peroxidase blocking solution prior to the addition of primary antibody (ab16502). After incubation with primary antibody overnight at 4°C, the slides were incubated in peroxidase-conjugated polymer.



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody (ab16502)

ICC/IF image of ab16502 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab16502, 1µg/ml) overnight at +4°C. The secondary antibody (green) was goat **anti-rabbit DyLight® 488** (IgG - H&L, pre-adsorbed) (**ab96899**) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1:200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-NF-kB p65 antibody (ab16502)

All lanes : Anti-NF-kB p65 antibody (ab16502) at 1 µg/ml

Lane 1 : Spleen (Mouse) Tissue Lysate

Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

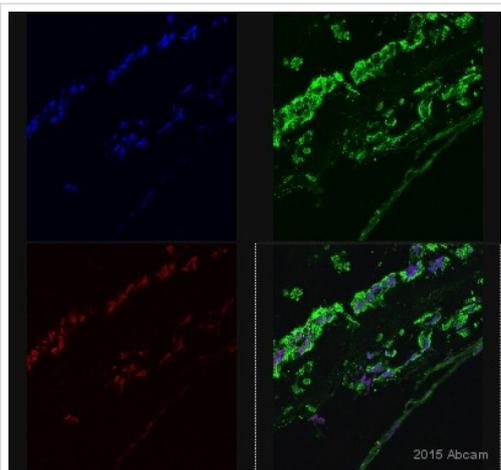
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 64 kDa

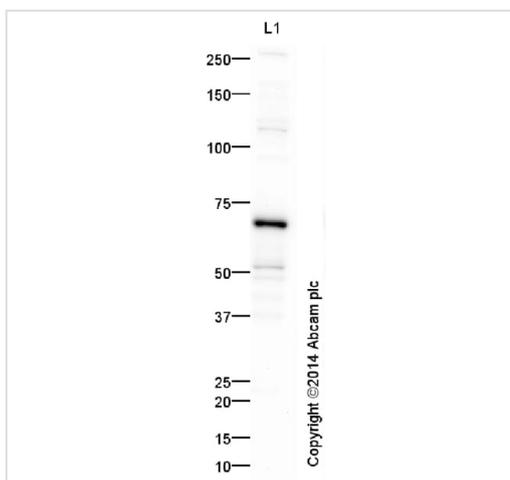
Exposure time: 8 minutes



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody (ab16502)

This image is courtesy of an anonymous abreview.

ab16502 staining NF-kB p65 in murine peritoneal tumour cells by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with paraformaldehyde and blocked with 1% BSA for 60 minutes at room temperature. Samples were incubated with primary antibody (1/1000) for 2 hours. An undiluted Alexa Fluor[®]647-conjugated goat anti-rabbit IgG polyclonal was used as the secondary antibody.



Western blot - Anti-NF-kB p65 antibody (ab16502)

Anti-NF-kB p65 antibody (ab16502) at 1 µg/ml + HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 50000 mg/ml

Developed using the ECL technique.

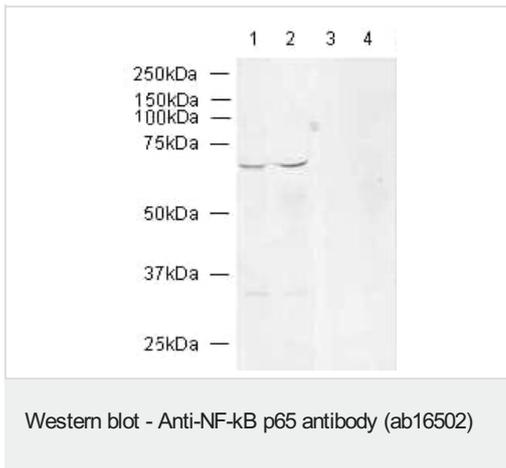
Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 64 kDa

Exposure time: 4 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab16502 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution [ab133406](#)



All lanes : Anti-NF-kB p65 antibody (ab16502) at 0.5 µg/ml

Lane 1 : HeLa whole cell lysate

Lane 2 : A431 whole cell lysate

Lane 3 : HeLa whole cell lysate with Human NF-kB p65 peptide (**ab16636**) at 1 µg/ml

Lane 4 : A431 whole cell lysate with Human NF-kB p65 peptide (**ab16636**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

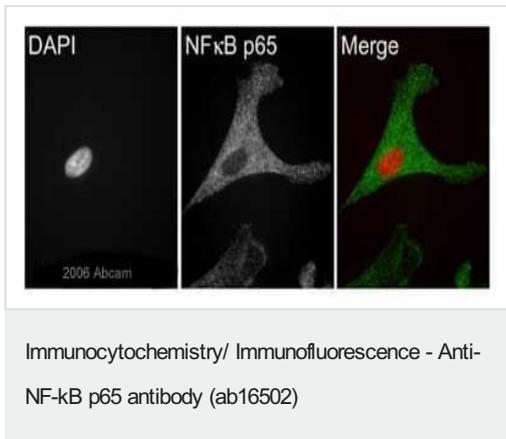
Secondary

All lanes : Alexa Fluor Goat polyclonal to Rabbit IgG at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 64 kDa



ab16502 at a 1/500 dilution staining Asynchronous and paraformaldehyde-fixed (4%) HeLa cells by immunocytochemistry.

The antibody was incubated with the cells 30 minutes and then detected using a Cy3 conjugated Goat Anti-Mouse IgG (H+L) antibody.

This image is courtesy of an Abreview by **Kirk McManus** submitted on **27 February 2006**.

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