abcam

ab307165

Anti-TNF alpha antibody [RM1005] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	RM1005
Purification technique	Affinity purification Protein A
Specificity	IHC-P is not suitable for the detection of normal tissues. Stimulation may be required to allow detection of the target protein due to low levels of endogenous expression in some samples. Please see images for recommended treatment conditions and positive controls.
Concentration	1.055 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ELISA

Tested

Species	Rat
Dilution info	-
Notes	-

Expected

Species	Mouse, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Mouse
Dilution info	-
Notes	Soluble form (17 kDa) and multimers of soluble form (33 kDa, 51 kDa) were also observed as described in literatures (PMID: 18523283, PMID: 28426652).

Species	Human
Dilution info	-
Notes	Soluble form (17 kDa) and multimers of soluble form (33 kDa, 51 kDa) were also observed as described in literatures (PMID: 18523283, PMID: 28426652).

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-

Notes	-

Species	Human
Dilution info	-
Notes	Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min followed by intracellularly stained with ab307164.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse, Human
Dilution info	-
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Rat, Mouse
Dilution info	-
Notes	-

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Rat, Mouse, Human
Dilution info	-
Notes	-

Target data

See full target information TNF

Function

Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation. Impairs regulatory T-cells (Treg) function in individuals with rheumatoid arthritis via FOXP3 dephosphorylation. Up-regulates the expression of protein phosphatase 1 (PP1), which dephosphorylates the key 'Ser-418' residue of FOXP3, thereby inactivating FOXP3 and rendering Treg cells functionally defective (PubMed:23396208). Key mediator of cell death in the anticancer action of BCG-stimulated neutrophils in combination with DIABLO/SMAC mimetic in the RT4v6 bladder cancer cell line (PubMed:16829952, PubMed:22517918, PubMed:23396208). Induces insulin resistance in adipocytes via inhibition of insulin-induced IRS1 tyrosine phosphorylation and insulin-induced glucose uptake. Induces GKAP42 protein degradation in adipocytes which is partially responsible for TNF-induced insulin resistance (By similarity). Plays a role in angiogenesis by inducing VEGF production synergistically with IL1B and IL6 (PubMed:12794819). Promotes osteoclastogenesis and therefore mediates bone resorption (By similarity). The TNF intracellular domain (ICD) form induces IL12 production in dendritic cells.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C

Notes

What are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

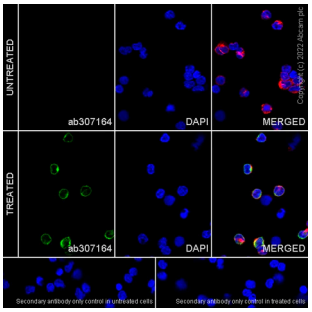
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

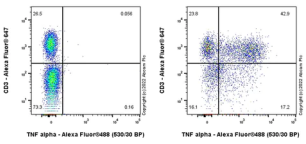
12 product images



Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Immunocytochemistry/ Immunofluorescence staining of Human PBMC (human peripheral blood mononuclear cell) using rabbit Anti-TNF alpha antibody

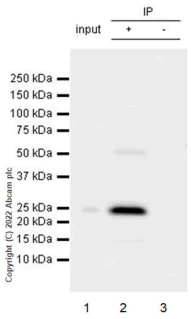
This data was developed using ab307164, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Human PBMC (human peripheral blood mononuclear cell) cells labeling TNF alpha with ab307164 at 1/500 (1.12 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing increased cytoplasmic staining in human PBMC treated with Phorbol-12-myristate-13-acetate (50ng/ml) and Ionomycin calcium (200ng/ml) and Brefeldin A (300ng/ml) for 4 hours. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 ug/ml) dilution (Red). The nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.



Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Flow Cytometry (Intracellular) staining of Human peripheral blood mononuclear cell (PBMC) treated with 50ng/ml PMA, 200ng/ml Ionomycin calcium and 300ng/ml BFA for 4h using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation. Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized Human peripheral blood mononuclear cell (PBMC) treated with 50ng/ml PMA, 200ng/ml Ionomycin calcium and 300ng/ml BFA for 4h (Right) / Untreated control (Left) cells labelling TNF alpha with ab307164 at 1/500 dilution (0.1ug)/ Left and Right (Red) compared with a isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Cells were surface stained with anti-CD3 conjugated to Alexa Fluor® 647. Then fixed with 2% PFA for 10min followed by intracellularly stained with ab307164.



Immunoprecipitation - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
TNF alpha was immunoprecipitated from 0.35 mg THP-1 (human monocytic leukemia monocyte) treated with 80nM TPA overnight, then treated with 100ng/ml LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate 5 ug with ab307164 at 1/30 dilution (2ug in 0.18mg lysates). Western blot was performed on the immunoprecipitate using ab307164 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: THP-1 (human monocytic leukemia monocyte) treated with 80nM TPA overnight, then treated with 100ng/ml LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate 5 ug

Lane 2: ab307164 IP in THP-1 treated with 80nM TPA overnight, then treated with 100ng/ml LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab307164 in THP-1 treated with 80nM TPA overnight, then treated with 100ng/ml LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds

Soluble form (17 kDa) and multimers of soluble form (33 kDa, 51 kDa) were also observed as described in literatures (PMID: 18523283, PMID: 28426652).

All lanes:
Immunoprecipitation - Anti-TNF alpha antibody [RM1005] (ab307164) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia monocyte) treated with 80nM TPA overnight, then treated with 100ng/ml LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate 5 µg

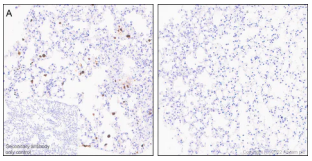
Lane 2:
THP-1 treated with 80nM TPA overnight, then treated with 100ng/ml LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 17 kDa, 26 kDa, 51 kDa

Exposure time: 6s



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

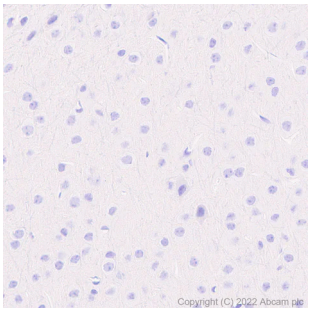
TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse lung using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded Mouse lung treated w tissue labeling TNF alpha with ab307164 at 1/1000 (0.56 ug/ml) followed by a ready to use LeicaDS9800 (Bond™

Polymer Refine Detection). Positive staining on mouse lung treated with LPS (1ug/ml for 16 hours) and BFA (1ug/ml for 16h hours) (Image A) and control mouse lung (Image B). The section was incubated with ab307164 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND™ RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



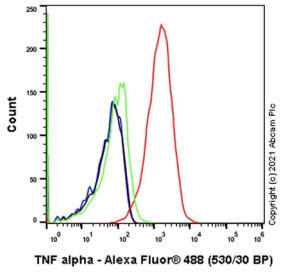
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse cerebrum using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling TNF alpha with ab307164 at 1/1000 (0.56 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Negative control: no staining on mouse cerebrum. The section was incubated with ab307164 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND™ RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

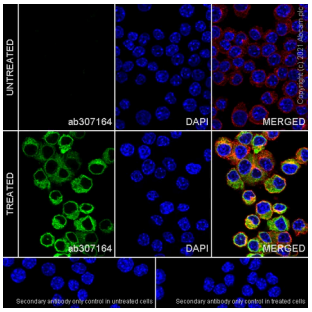
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Flow Cytometry (Intracellular) - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Flow Cytometry (Intracellular) staining of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 3h and add 1µg/ml BFA for another 3h using rabbit Anti-TNF alpha antibody

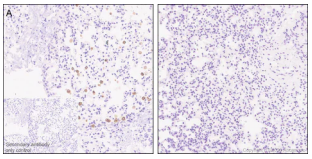
This data was developed using ab307164, the same antibody clone in a different buffer formulation. Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 3h and add 1µg/ml BFA for another 3h (Red) / Untreated control (Green) cells labelling TNF alpha with ab307164 at 1/500 dilution (0.1ug) (Red) and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Immunocytochemistry/ Immunofluorescence staining of RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation. Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) cells labeling TNF alpha with ab307164 at 1/500 (1.12 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing increased cytoplasmic staining in RAW 264.7 treated with lipopolysaccharide (100 ng/ml) and Brefeldin A (1 µg/ml) for 3 hours.Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 ug/ml) dilution (Red). The nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

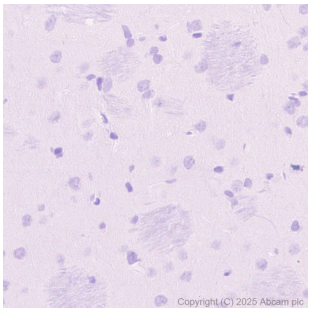


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of formalin fixed paraffin embedded rat lung labelling TNF alpha with ab307164 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [RM1005] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin.

Positive staining on rat lung treated with LPS (1ug/ml for 16 hours) and BFA (1ug/ml for 16h hours) (Image A) and no staining on wild type rat lung (Image B).

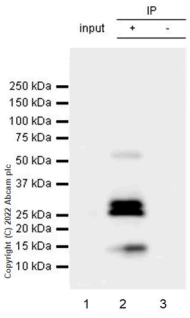


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Rat cerebrum using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of formalin fixed paraffin embedded rat cerebrum labelling TNF alpha with ab307164 at a concentration of 1/1000 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins. The primary antibody Anti-TNF alpha antibody [RM1005] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin.

Negative control: no staining on rat cerebrum.



Immunoprecipitation - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
TNF alpha was immunoprecipitated from 0.35 mg RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate 10 ug with ab307164 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab307164 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate 10 ug

Lane 2: ab307164 IP in RAW 264.7 treated with 100ng/ml LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab307164 in RAW 264.7 treated with 100ng/ml LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 6 seconds

Soluble form (17 kDa) and multimers of soluble form (33 kDa, 51 kDa) were also observed as described in literatures (PMID: 18523283, PMID: 28426652).

All lanes:
Immunoprecipitation - Anti-TNF alpha antibody [RM1005] (ab307164) at 1/1000 dilution

Lane 1:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 100ng/ml LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate 10 µg

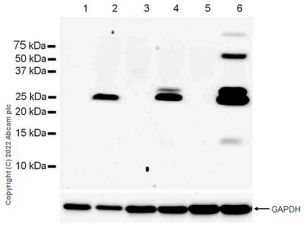
Lane 2:
RAW 264.7 treated with 100ng/ml LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 17 kDa, 26 kDa, 33 kDa, 51 kDa

Exposure time: 6s



Western blot - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

TNF alpha Western blot staining using rabbit Anti-TNF alpha antibody

This data was developed using ab307164, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Soluble form (17 kDa) and multimers of soluble form (33 kDa, 51 kDa) were also observed as described in literatures (PMID: 18523283, PMID: 28426652).
All lanes:
Western blot - Anti-TNF alpha antibody [RM1005] (ab307164) at 1/1000 dilution

Lane 1:
THP-1 (human monocytic leukemia monocyte) whole cell lysate 20 µg

Lane 2:
THP-1 treated with 80nM TPA overnight, then treated with 100 ng/mL LPS for 3h and add 300ng/ml BFA for another 3h whole cell lysate 20 µg

Lane 3:
RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate 20 µg

Lane 4:
RAW 264.7 treated with 100 ng/mL LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate 20 µg

Lane 5:
NR8383 (rat alveolar macrophage) whole cell lysate 20 µg

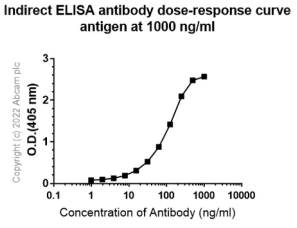
Lane 6:
NR8383 treated with 100 ng/mL LPS for 4h and add 300ng/ml BFA for another 3h whole cell lysate 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 17 kDa, 26 kDa, 33 kDa, 51 kDa

Exposure time: 180s



Indirect ELISA - Anti-TNF alpha antibody [RM1005] - BSA and Azide free (ab307165)

Indirect ELISA analysis of ab173305 at 1/1000 ng/ml. The secondary antibody used was Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution. Substrate solution: p-nitrophenyl phosphate(PNPP).

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.