Product datasheet

Human TNF alpha ELISA Kit ab181421

Overview

Product name
Human TNF alpha ELISA Kit

Detection method
Colorimetric

Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC media</td>
<td>8</td>
<td></td>
<td>2.3%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC media</td>
<td>3</td>
<td></td>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>

Sample type
Cell culture supernatant, Serum, Heparin Plasma, EDTA Plasma, Citrate Plasma

Assay type
Sandwich (quantitative)

Sensitivity
14 pg/ml

Range
31.25 pg/ml - 2000 pg/ml

Recovery

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>99.5</td>
<td>80% - 119%</td>
</tr>
<tr>
<td>Cell culture media</td>
<td>100.97</td>
<td>95% - 109%</td>
</tr>
<tr>
<td>Heparin Plasma</td>
<td>98</td>
<td>91% - 106%</td>
</tr>
<tr>
<td>EDTA Plasma</td>
<td>102</td>
<td>95.7% - 110%</td>
</tr>
<tr>
<td>Citrate Plasma</td>
<td>93.09</td>
<td>83.5% - 99%</td>
</tr>
</tbody>
</table>

Assay time
1h 30m
Assay duration
One step assay

Species reactivity
Reacts with: Human
Does not react with: Mouse

Product overview
Abcam’s TNF-alpha *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of TNF-alpha protein in Human serum, plasma and culture media.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Notes
TNF-alpha, also known as cachectin or TNFSF1A, is the prototypic ligand of the TNF superfamily which plays a central role in inflammation, apoptosis, proliferation, invasion, angiogenesis, metastasis and morphogenesis. It is expressed on macrophages, endothelial, epithelial and tumor cells as a 26kDa transmembrane protein. TNF-alpha is cleaved by proteolytic processing into six chains: (1) TNF membrane form, (2) Intracellular domain 1, (3) Intracellular domain 2, (4) C-domain 1, (5) C-domain 2 and (6) TNF soluble form. Signaling from TNF-alpha differs depending on the type of ligand initiating the signaling event (intracellular, membrane or soluble). As an example, the membrane form of TNF-alpha appears to mediate anti-tumorigenic therapeutic responses whereas the soluble ligand is linked to inflammation and proliferation.

Tested applications
Suitable for: Sandwich ELISA

Platform
Microplate

Properties

Storage instructions
Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Wash Buffer PT (ab206977)</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>ab221825 - Antibody Diluent 5BI</td>
<td>1 x 6ml</td>
</tr>
<tr>
<td>Plate Seals</td>
<td>1 unit</td>
</tr>
<tr>
<td>Sample Diluent 50BP</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>Sample Diluent NS (ab193972)</td>
<td>1 x 50ml</td>
</tr>
<tr>
<td>SimpleStep Pre-Coated 96-Well Microplate (ab206978)</td>
<td>1 unit</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 12ml</td>
</tr>
</tbody>
</table>
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.

Involvement in disease

Genetic variations in TNF are a cause of susceptibility psoriatic arthritis (PSORAS) [MIM:607507]. PSORAS is an inflammatory, seronegative arthritis associated with psoriasis. It is a heterogeneous disorder ranging from a mild, non-destructive disease to a severe, progressive, erosive arthropathy. Five types of psoriatic arthritis have been defined: asymmetrical oligoarthritis characterized by primary involvement of the small joints of the fingers or toes; asymmetrical arthritis which involves the joints of the extremities; symmetrical polyarthritis characterized by a rheumatoidlike pattern that can involve hands, wrists, ankles, and feet; arthritis mutilans, which is a rare but deforming and destructive condition; arthritis of the sacroiliac joints and spine (psoriatic spondylitis).

Sequence similarities

Belongs to the tumor necrosis factor family.

Post-translational modifications

The soluble form derives from the membrane form by proteolytic processing. The membrane form, but not the soluble form, is phosphorylated on serine residues. Dephosphorylation of the membrane form occurs by binding to soluble TNFRSF1A/TNFR1. O-glycosylated; glycans contain galactose, N-acetylgalactosamine and N-acetylneuraminic acid.

Cellular localization

Secreted and Cell membrane.

Applications

Our Abpromise guarantee covers the use of ab181421 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandwich ELISA</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

Images
Background-subtracted data values (mean +/- SD) are graphed.

Example of TNF-alpha standard curve

Background-subtracted data values (mean +/- SD) are graphed.

Example of TNF-alpha standard curve

Human PBMCs were cultured in RPMI supplemented with 10% fetal calf serum, 2mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were cultured for 2 days at 37°C in the presence or absence of PHA. The concentrations of TNF-alpha were interpolated from the calibration curve and corrected for sample dilution. The mean TNF-alpha concentration was determined to be undetectable in basal media, 1.2 ng/mL in unstimulated PMBC supernatants and 4.7 ng/mL in stimulated PBMC supernatants.

Specificity of TNF-alpha signal on stimulated and non stimulated media supernatants.

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