

# Product Datasheet

## Recombinant Human TNF-alpha Pro form Protein NB1012-PS

Unit Size: 10 ug

Protect from light. Use a manual defrost freezer and avoid repeated freeze-thaw cycles. 12 months from date of receipt, -20 to -70 °C as supplied. 3 months, -20 to -70 °C under sterile conditions after opening.

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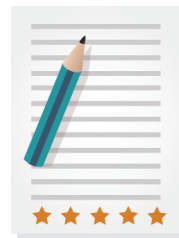
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**NB1012-PS**

Recombinant Human TNF-alpha Pro form Protein

Product Information	
Unit Size	10 ug
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Protect from light. Use a manual defrost freezer and avoid repeated freeze-thaw cycles. 12 months from date of receipt, -20 to -70 °C as supplied. 3 months, -20 to -70 °C under sterile conditions after opening.
Reconstitution Instructions	Reconstitute at 200 ug/mL in sterile, deionized water.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Buffer	0.2 um filtered solution in Urea, NaCl, NaH <sub>2</sub> PO <sub>4</sub> and DTT
Target Molecular Weight	45 kDa

**Product Description**

<b>Description</b>	<p>Recombinant Human Pro TNF-a Bacterial Protein Fusion Partner protein</p> <p><b>Source:</b> <i>E. coli</i> derived</p> <p><b>Activity</b> Measured by its ability to be used as a protein substrate for TACE/ADAM17. Under the described conditions TACE/ADAM17 will cleave pro-TNF-a to produce mature TNF-a.</p> <p><b>Assay Procedure</b></p> <p>Materials:</p> <ul style="list-style-type: none"> <li>• Assay Buffer: 50 mM Tris, pH 8.0</li> <li>• Recombinant Human Pro TNF-a Fusion Protein (rhPro-TNF-a) (Catalog # 1012-PS)</li> <li>• Recombinant Human TACE/ADAM17 (rhTACE) (Catalog # <a href="#">930-ADB</a>)</li> <li>• Positive Control: Recombinant Human TNF-a (rhTNF-a) (Catalog # <a href="#">210-TA</a>)</li> <li>• Goat Anti-Human TNF-a Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # <a href="#">BAF210</a>)</li> <li>• SDS-PAGE followed by Western Blotting</li> </ul> <ol style="list-style-type: none"> <li>1. Dilute rhPro-TNF-a to 0.2 mg/mL in Assay Buffer.</li> <li>2. Dilute rhTACE to 0.2 mg/mL in Assay Buffer.</li> <li>3. Prepare the following vials for a final volume of 20 uL.             <ol style="list-style-type: none"> <li>i. 10 uL of rhPro-TNF-a at 0.2 mg/mL + 10 uL of rhTACE at 0.2 mg/mL</li> <li>ii. 10 uL of rhPro-TNF-a at 0.2 mg/mL + 10 uL of Assay Buffer (control-with incubation)</li> <li>iii. 10 uL of rhPro-TNF-a at 0.2 mg/mL + 10 uL of Assay Buffer (control-without incubation)</li> </ol> <p>Note: At this point the concentration of rhPro-TNF-a in the reaction tubes is 0.1 mg/mL.</p> </li> <li>4. Incubate vials at 37C overnight (except for the non-incubated control, store at =-20C).</li> <li>5. Stop the reactions by adding the reducing gel loading buffer for SDS-PAGE to all vials. Heat the samples at 100C for 3-5 minutes.             <ol style="list-style-type: none"> <li>i. Dilute the reaction tubes (and control without incubation) to 50 ng/15 uL in reducing sample buffer.</li> </ol> </li> <li>6. Prepare a sample of Positive Control at 5 ng/15 uL in reducing sample buffer. Heat the sample at 100C for 3-5 minutes.</li> <li>7. Load the samples on a 15% gel.             <ol style="list-style-type: none"> <li>i. 50 ng/lane (15 uL) of rhPro-TNF-a of the incubated reactions (including the control with incubation).</li> <li>ii. 50 ng/lane (15 uL) of rhPro-TNF-a of the control (without incubation).</li> <li>iii. 5 ng/lane (15 uL) of Positive Control (Catalog # 210-TA).</li> </ol> </li> <li>8. Follow SDS-PAGE/<a href="#">Western Blotting</a> procedures.             <ol style="list-style-type: none"> <li>i. Use the Biotinylated Anti-human TNF-a/TNFSF1A antibody at 0.1 ug/mL. (Catalog # <a href="#">AF-210-NA</a> may be used in place of # BAF210.)</li> </ol> </li> <li>9. Visually determine processing of rhPro-TNF-a to the mature form by rhTACE by comparing the incubated reactions to the Positive Control.</li> </ol>
<b>Gene ID</b>	7124
<b>Gene Symbol</b>	TNF
<b>Species</b>	Human
<b>Endotoxin Note</b>	<1.0 EU per 1 ug of the protein by the LAL method.

## Procedures

### Protocol specific for TNF-alpha (NB1012-PS)

Activity Measured by its ability to be used as a protein substrate for TACE/ADAM17. Under the described conditions TACE/ADAM17 will cleave pro-TNF-a to produce mature TNF-a.

Assay Procedure

Materials:

Assay Buffer: 50 mM Tris, pH 8.0

Recombinant Human Pro TNF-a Fusion Protein (rhPro-TNF-a) (Catalog # 1012-PS)

Recombinant Human TACE/ADAM17 (rhTACE) (Catalog # [930-ADB](http://www.rndsystems.com/product_results.aspx?k=930-ADB))

Positive Control: Recombinant Human TNF-a (rhTNF-a) (Catalog # [210-TA](http://www.rndsystems.com/product_results.aspx?k=210-TA))

Goat Anti-Human TNF-a Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # [BAF210](http://www.rndsystems.com/product_results.aspx?k=BAF210))

SDS-PAGE followed by Western Blotting

Dilute rhPro-TNF-a to 0.2 mg/mL in Assay Buffer.

Dilute rhTACE to 0.2 mg/mL in Assay Buffer.

Prepare the following vials for a final volume of 20 uL.

10 uL of rhPro-TNF-a at 0.2 mg/mL + 10 uL of rhTACE at 0.2 mg/mL

10 uL of rhPro-TNF-a at 0.2 mg/mL + 10 uL of Assay Buffer (control-with incubation)

10 uL of rhPro-TNF-a at 0.2 mg/mL + 10 uL of Assay Buffer (control-without incubation) **Note:** At this point

the concentration of rhPro-TNF-a in the reaction tubes is 0.1 mg/mL.

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<br/>Incubate vials at 37C overnight (except for the non-incubated control, store at =-20C).

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<br/>Stop the reactions by adding the reducing gel loading buffer for SDS-PAGE to all vials. Heat the samples at 100C for 3-5 minutes.

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<br/>Dilute the reaction tubes (and control without incubation) to 50 ng/15 uL in reducing sample buffer.

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<br/>Prepare a sample of Positive Control at 5 ng/15 uL in reducing sample buffer. Heat the sample at 100C for 3-5 minutes.

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<br/>Load the samples on a 15% gel.

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<br/>50 ng/lane (15 uL) of rhPro-TNF-a of the incubated reactions (including the control with incubation).

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<br/>50 ng/lane (15 uL) of rhPro-TNF-a of the control (without incubation).

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<br/>5 ng/lane (15 uL) of Positive Control (Catalog # 210-TA).

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<br/>Follow SDS-PAGE/<A class=NoLineLink href="http://www.rndsystems.com/literature\_qc\_protocol.aspx">Western Blotting</A> procedures.

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<br/>Use the Biotinylated Anti-human TNF-a/TNFSF1A antibody at 0.1 ug/mL. (Catalog # <A class=NoLineLink href="http://www.rndsystems.com/product\_results.aspx?k=AF-210-NA">AF-210-NA</A> may be used in place of # BAF210.)

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<br/>Visually determine processing of rhPro-TNF-a to the mature form by rhTACE by comparing the incubated reactions to the Positive Control.

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