

NB1012-PS Protocol**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.

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

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.

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

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

Load the samples on a 15% gel.

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

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

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

Follow SDS-PAGE/[Western Blotting](http://www.rndsystems.com/literature_qc_protocol.aspx) procedures.

Use the Biotinylated Anti-human TNF-a/TNFSF1A antibody at 0.1 ug/mL. (Catalog # [AF-210-NA](http://www.rndsystems.com/product_results.aspx?k=AF-210-NA) may be used in place of # BAF210.)

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