

NB100-105L Protocol

Antibody Competition protocol for HIF-1 alpha Antibody (NB100-105L)

Materials

Assay Buffer: 50 mM Tris, pH 8.0

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

Recombinant Human TACE/ADAM17 (rhTACE) (Catalog # 930-ADB)

Positive Control: Recombinant Human TNF-alpha (rhTNF-alpha) (Catalog # 210-TA)

Goat Anti-Human TNF alpha Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # BAF210)

SDS-PAGE followed by Western Blotting

- 1) Dilute rhPro-TNF-alpha to 0.2 mg/mL in Assay Buffer.
- 2) Dilute rhTACE to 0.2 mg/mL in Assay Buffer.
- 3) Prepare the following vials for a final volume of 20 uL.
 - a) 10 uL of rhPro-TNF-alpha at 0.2 mg/mL + 10 uL of rhTACE at 0.2 mg/mL
 - b) 10 uL of rhPro-TNF-alpha at 0.2 mg/mL + 10 uL of Assay Buffer (control-with incubation)
 - c) 10 uL of rhPro-TNF-alpha at 0.2 mg/mL + 10 uL of Assay Buffer (control-without incubation)

Note: At this point the concentration of rhPro-TNF-alpha in the reaction tubes is 0.1 mg/mL.
- 4) Incubate vials at 37 degrees C overnight (except for the non-incubated control, store at less than or equal to 20 degrees C).
- 5) Stop the reactions by adding the reducing gel loading buffer for SDS-PAGE to all vials. Heat the samples at 100 degrees C for 3-5 minutes.
 - a) Dilute the reaction tubes (and control without incubation) to 50 ng/15 uL in reducing sample buffer.
- 6) Prepare a sample of Positive Control at 5 ng/15 uL in reducing sample buffer. Heat the sample at 100 degrees C for 3-5 minutes.
- 7) Load the samples on a 15% gel.
 - a) 50 ng/lane (15 uL) of rhPro-TNF-alpha of the incubated reactions (including the control with incubation).
 - b) 50 ng/lane (15 uL) of rhPro-TNF-alpha of the control (without incubation).
 - c) 5 ng/lane (15 uL) of Positive Control (Catalog # 210-TA).
- 8) Follow SDS-PAGE/Western Blotting procedures.
 - a) Use the Biotinylated Anti-human TNF-alpha /TNFSF1A antibody at 0.1 ug/mL. (Catalog # AF-210-NA may be used in place of # BAF210.)
- 9) Visually determine processing of rhPro-TNF-alpha to the mature form by rhTACE by comparing the incubated reactions to the Positive Control.

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