

Anti-HA-tag mAb-Biotin

CODE No. M180-6

CLONALITY Monoclonal
CLONE TANA2
ISOTYPE Mouse IgG2b κ
QUANTITY 50 μ L

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH conjugated synthetic peptide, YPYDVPDYA (HA-tag)
FORMULATION PBS (pH 7.2) containing 1% BSA and 0.09% NaN_3

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

Sandwich ELISA 1:2,000 for chemiluminescence detection system

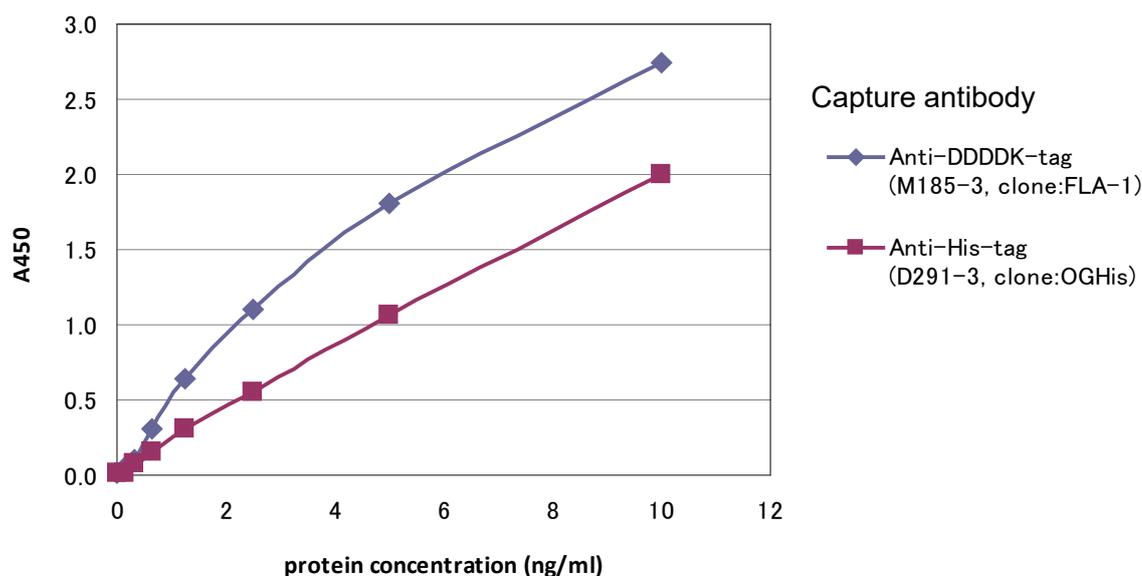
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Sandwich ELISA

- 1) Add 100 μL /well of 5 $\mu\text{g}/\text{mL}$ capture antibody diluted 0.1 M Carbonate buffer (pH 9.6) to the 96-well plate. Incubate overnight at 4°C.
- 2) Wash the plate with PBS (2 times).
- 3) Add 200 μL /well of Blocking Buffer (1% BSA/5% Sucrose/0.15% Proclin150/PBS). Incubate for 1 hr. at room temperature.
- 4) Discard the Blocking Buffer. Add 100 μL /well of HA-tagged protein (His-DDDDK-V5-HA-Myc-monomeric Azami-Green) in Assay diluent (1% BSA/0.1% Tween-20/0.15% Proclin150/PBS) to each well.
- 5) Incubate for 1 hr. at room temperature.
- 6) Wash the plate with PBS-T [0.05% Tween-20 in PBS] (4 times).
- 7) Add 100 μL /well of anti-HA-tag mAb-Biotin (MBL; code no. M180-6) diluted with Assay diluent as suggested in the **APPLICATION**. Incubate for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 8) Wash the plate with PBS-T (4 times).
- 9) Add 100 μL /well of 1:40,000 streptavidin-HRP (GE Healthcare; code no. RPN4401) diluted with SA-HRP diluent (20 mM HEPES/1% BSA/0.135 M NaCl). Incubate for 30 min. at room temperature.
- 10) Wash the plate with PBS-T (4 times).
- 11) Add 100 μL /well of substrate solution (ex. TMB). Incubate for appropriate time at room temperature.
- 12) Add 100 μL /well of stop solution (ex. 1 M H_2SO_4).
- 13) Read absorbance at 450 nm.



ELISA for measurement of HA-tagged protein

Capture antibody:

Anti-DDDDK-tag mAb (MBL; code no. M185-3)

Anti-His-tag mAb (MBL; code no. D291-3)

Detector antibody:

Anti-HA-tag mAb-Biotin (MBL; code no. M180-6)

Sample:

His-DDDDK-V5-HA-Myc-monomeric Azami-Green