APH650Hu01 100μg
Active Melanoma Inhibitory Activity Protein 1 (MIA1)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Gly25~Gln131
Tags: N-terminal His-tag
Purity: >80%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 8.7
Predicted Molecular Mass: 16.4kDa
Accurate Molecular Mass: 16kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

GPMPKL ADRKLCADQE CSHPISMAVA LQDYMAPDCR FLTIHRGQVV YVFSKLKGRG RLFWGGSVQG DYYGDLAARL GYFPSSIVRE DQTLKPGKVD VKTDKWDFYC Q

[ACTIVITY]

Melanoma inhibitory activity (MIA), also known as cartilage-derived retinoic acid-sensitive protein (CD-RAP), is a 12-kDa protein that is secreted from both chondrocytes and malignant melanoma cells. MIA has been reported to have effects on cell growth and adhesion, and it may play a role in melanoma metastasis and cartilage development. In the presence of MIA, we observed enhanced migratory ability of melanocytic cells, induction of melanoma-associated genes as well as inhibition of apoptosis due to anoikis. S100 Calcium Binding Protein B (S100B) is a high affinity ligand for MIA1, a functional binding ELISA assay was conducted to detect the interaction of recombinant human MIA1 and recombinant bovine S100B. Briefly, MIA1 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to S100B-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST and incubated for 1h with anti-MIA1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37℃, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant humant MIA1 and recombinant bovine S100B was shown in Figure 1, the EC50 for this effect is 1.1 ug/mL.

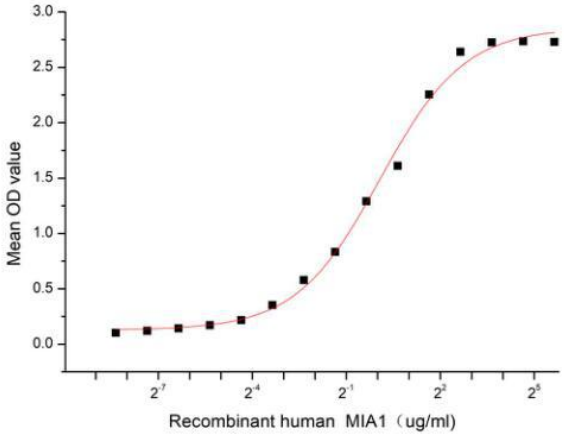
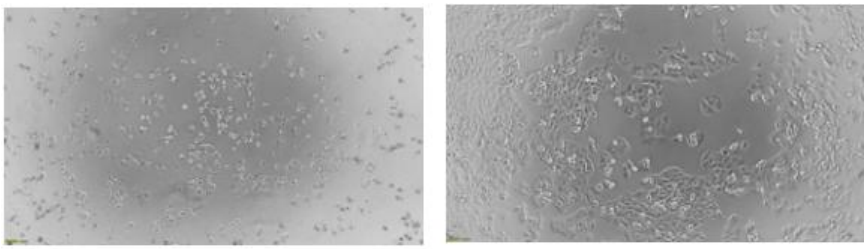


Figure 1. The binding activity of recombinant humant MIA1 and recombinant bovine S100B

To test the effect of MIA1 on cell apoptosis, A375 cells were seeded into triplicate wells of 96-well plates at a density of 4,000 cells/well and allowed to attach overnight, then the medium was replaced with various concentrations of recombinant human MIA1 diluted with 5% serum standard DMEM. After incubated for 48h, cells were observed by inverted microscope and cell viability was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37 ℃. Apoptosis of A375 cells after incubation with MIA1 for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 assay after incubation with recombinant human MIA1 for 48h. The result was shown in Figure 2. It was obvious that MIA1 significantly decreased cell viability of A375 cells. The ED50 of recombinant human MIA1 is 0.53 ug/ml.



A B

Figure 2. Inhibition of A375 cells proliferation after stimulated with recombinant human MIA1

(A) A375 cells cultured in DMEM, stimulated with 1.25 μg/mL MIA1 for 48h;

(B) Unstimulated A375 cells cultured in DMEM for 48h.

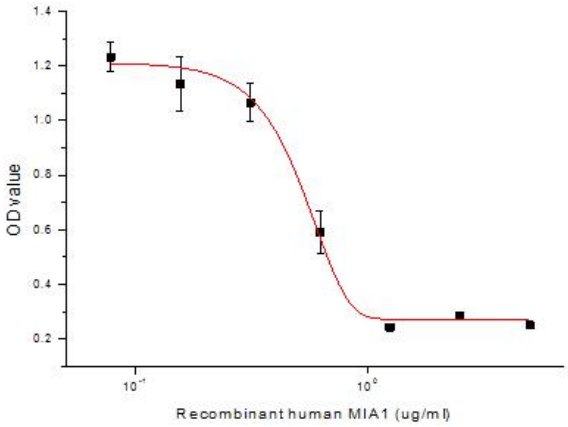


Figure 3. Inhibition of A375 cells proliferation after stimulated with recombinant human MIA1

[IDENTIFICATION]

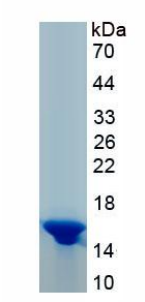


Figure 4. SDS-PAGE

Sample: Active recombinant MIA1, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.