Human MIA2
ELISA Development Kit (ABTS)

Catalog No.: LS-F31271

DESCRIPTION

This kit includes the 4 principal components required to prepare approximately 1000 sandwich ELISA plate wells for the quantitative measurement of Human MIA2 within the range of 16–2000 pg/ml; the capture and detection antibodies, an antigen standard with which to create a standard curve and the avidin-HRP conjugate for detection. Below you will find our recommendations for all other materials needed, such as commercial reagents, recipes for solution preparation, instructions for coating the plates, and a recommended ELISA protocol.

Target Synonyms: MIA2, melanoma inhibitory activity 2, Melanoma inhibitory activity 2

RECONSTITUTION & STORAGE

Capture Antibody (lyophilized)**:** 100 µg of Rabbit anti-MIA2 capture antibody is supplied lyophilized with 2.5 mg D-mannitol. Centrifuge the vial prior to opening, then reconstitute with 1000 µl sterile water for a concentration of 100 µg/ml.

Biotinylated Detection Antibody (lyophilized)**:** 25 µg of biotinylated Rabbitanti-MIA2 antibody is supplied lyophilized with 2.5 mg D-Mannitol. Centrifuge the vial prior to opening, then reconstitute with 250 µl sterile water for a concentration of 100 µg/ml.

Human MIA2 Standard (lyophilized)**:** 1 µg of recombinant MIA2 protein is supplied lyophilized with 2.2 mg BSA and 11 mg D-mannitol. To prepare a 1µg/ml Standard Stock Solution, centrifuge the vial prior to opening, and reconstitute with 1000 µl sterile water.

> ***Note****: Reconstituted antibodies and standards are stable for 2 weeks at 2-8°C or for up to 6 months at -20°C.*

Avidin-HRP Conjugate: The Avidin-HRP Conjugate is supplied as 60 µl liquid. Upon receipt, centrifuge the vial prior to opening and prepare ten 6µl aliquots for storage at -20°C for up to 2 years from date of receipt. Once thawed do not refreeze. **Use Avidin-HRP Conjugate with ABTS only.**

RECOMMENDED MATERIALS

- ABTS Liquid Substrate Solution (Sigma Cat. # A3219)
- BSA (Sigma Cat # A-7030)
- Dulbecco's PBS [10x] (Gibco BRL Cat. # 14200-075)
- ELISA Plates, Strips (Nunc MaxiSorp Cat. #439454)
- Tween-20 (Sigma Cat. # P-7949)

RECOMMENDED SOLUTIONS

All solutions should be at ambient temperature prior to use.

- PBS: dilute 10x PBS to 1x PBS, pH 7.2 in sterile water
- Wash Buffer: 0.05% Tween-20 in PBS
- Block Buffer*: 1% BSA in 1x PBS
- Diluent*: 0.05% Tween-20, 0.1% BSA in 1x PBS

  * Sterile filter and store at 4°C for up to 1 week.

PLATE PREPARATION

1. Dilute the Capture Antibody to a concentration of 0.5 µg/ml with 1x PBS and immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Invert the plate on a paper towel to remove residual buffer.
3. Add 300 µl of Block Buffer to each well and incubate for at least 1 hour at room temperature.
4. Aspirate each well and wash 4 times using 300 µl of Wash Buffer.

ELISA PROTOCOL

5. **Standard/ Sample:** Using Diluent, prepare a serial dilution series of the MIA2 Standard Stock Solution from 2000 pg/ml to 16 pg/ml. Use Diluent alone as a 0 pg/ml negative control. Researchers must determine the optimal samples dilutions for their particular experiment. Using Diluent, prepare a dilution series of your unknown sample. Add 100 µl of standard or sample to each well in triplicate and incubate at room temperature for at least 2 hours.
6. **Detection:** Dilute the desired amount of Detection Antibody to a concentration of 0.25 µg/ml with Diluent. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Add 100 µl of Detection Antibody per well and incubate at room temperature for 2 hours.
7. **Avidin-HRP Conjugate:** For each plate dilute 5.5 µl of Avidin-HRP Conjugate with 11 ml of Diluent. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Add 100 µl of Avidin-HRP Conjugate to each well and incubate 30 minutes at room temperature.
8. **ABTS Liquid Substrate:** ABTS Liquid Substrate Solution should be at ambient temperature prior to use. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Add 100 µl of ABTS Liquid Substrate Solution to each well and incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 405nm with wavelength correction set at 650nm. The plate should be monitored at 5-minute intervals for approximately 50 minutes. Reliable standard curves are obtained when the O.D. reading does not exceed 0.2 units for the zero standard concentrations, or 1.4 units for the highest standard concentration.
9. Use the O.D. values of the antigen standard serial dilution to generate a Standard Curve. This Standard Curve can then be used to calculate the concentration of the antigen in the unknown sample based on its O.D. value. See an example Standard Curve on the back side of this document. A Standard Curve should always be determined within each experiment.

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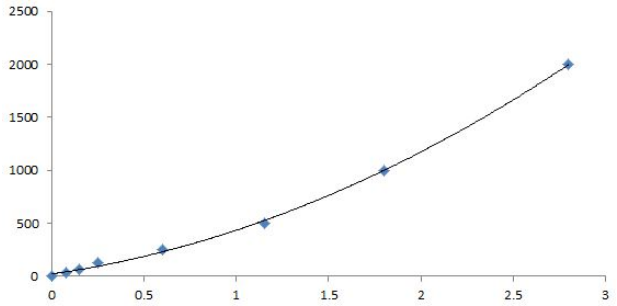
Tested Reactivity:
When tested at 50ng/ml the following antigens did not exhibit significant cross reactivity: Human MIA, OTOR.

Example Standard Curve: This LS-F31271 standard curve is an example only and may not be representative of this specific lot.



Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

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LSBio
LifeSpan BioSciences, Inc.

2 Shaker Rd Suite B101 Shirley, MA 01464
Tel: 206.374.1102
Fax: 206.577.4565
Technical.Support@LSBio.com