Human Anti-Muscarinic Cholinergic Receptor 1 (M1)-Antibodies

Catalog Number:
M1A31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0: 07/2018

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INTENDED USE

Muscarinic cholinergic receptors (mAChRs) are acetylcholine receptors that form G protein-receptor complexes in the cell membranes of certain neurons and other cells. The CellTrend anti-muscarinic cholinergic receptor 1 (M1)-Antibody EIA is designed for the determination of antibodies against the muscarinic cholinergic receptor 1 (M1) in serum.

PRINCIPLE OF THE ASSAY

The CellTrend muscarinic cholinergic receptor 1 (M1) ELISA assay kit is an antibody screening test. M1 receptor has been pre-coated onto a microtiter plate. During the first incubation the anti-muscarinic cholinergic recetor 1-antibodies of the samples are immobilized on the plate. The autoantibodies are detected with an HRP labeled anti-human IgG antibody. In the following enzymatic substrate reaction, the intensity of the color correlates with the concentration and/or avidity of anti-muscarinic cholinergic receptor 1 antibodies.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8 °C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. If crystals have formed, mix gently until the crystals have completely dissolved. Reagents from different kit lot numbers should not be combined or interchanged.

1. **The microplate strips** MTP are ready to use. Remove excess strips (breakable) from the frame, reseal in the bag with the desiccant and store at 2-8°C.
2. **Dilute the wash buffer** BUF WASH 10x with deionized or distilled water **1:10** (e.g. 50 ml + 450 ml water). The dilution solution is stable for 30 days at 2-8°C.
3. **Dilute the HRP conjugate** CONJ ENZ 100x with diluent DIL Conj. **1:100**. The required amount of conjugate should be prepared freshly.
4. **Dilute the human serum samples** with diluent DIL SPE **1:100**. Store undiluted samples at -20°C.
5. **The Controls** CONTROL, the diluent sample DIL SPE and the diluent conjugate DIL Conj are ready to use.
6. **Standards** CAL 1-6, are ready to use.

SAFETY PRECAUTIONS

- This assay procedure should be carried out only by qualified and well-trained employees.
- Lipaemic, icteric, haemolysed or microbially contaminated specimen may cause interference.
- Do not mix reagents from different lots
- Some components of the kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Some components of this kit contain Thirmerosal, a mercury containing compound. Follow routine precautions for handling hazardous chemicals. Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see materials provided). Wash thoroughly with water in case of contact and possibly look up a doctor.

- The stop solution contains 0.5M sulfuric acid. Wash thoroughly with water in case of contact with skin. In case of contact with eyes rinse with much water and look up a doctor.
- Do not allow wells to become dry once assay has begun.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision single channel pipettes capable of delivering 50 µL, 100 µL, and 1000 µL etc.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass tubes.
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

ASSAY PROCEDURE

It is recommended that all samples and standards be assayed in duplicates.

1. Prepare all reagents and samples as directed above.
2. Pipette 100 µl of dilution samples, standards, control or diluent DIL SPE (as blank) into well.
3. Seal wells with adhesive strip and incubate for 2 hours at 4°C temperature.
4. Aspirate fluid from wells and wash three times with 300 µl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Dispense 100 µl of diluted HRP conjugate into each well.
6. Seal wells with adhesive strip and incubate for 1 hour (with shaking) at room temperature.
7. Repeat the wash as in step 4.
8. Dispense 100 µl of the TMB substrate SUBS TMB solution into each well.
9. Incubate for 20 minutes at room temperature in the dark.
10. Add 100 µl of stop solution SOLN STOP to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

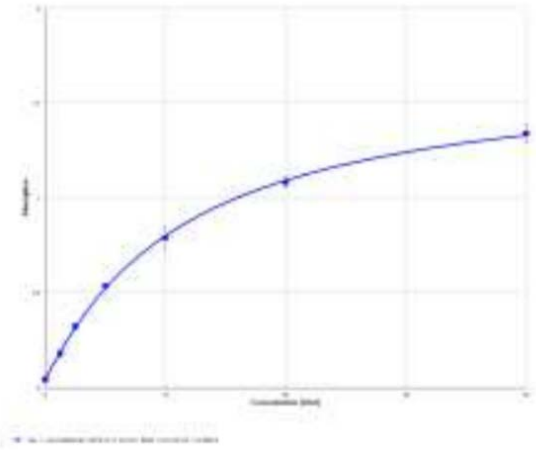
INTERPRETATION OF RESULTS

Create a standard curve using computer software capable of generating a curve fit (four parameter fit; x-axis: linear, anti-M1R-Ab standard points (1.25 U/ml, 2.5 U/ml, 5 U/ml, 10 U/ml, 20 U/ml, 40 U/ml); y-axis: linear, absorbance). The sample concentrations can be calculated from the standard curve.

A run is considered valid if the controls are within the expected range

We recommend that each laboratory establish its own range for the population tested.

Samples over the standard curve can be assayed again using a higher dilution factor (e.g 1:500). In this case the concentration read from the standard curve must be multiplied by the additional dilution factor (e.g 5 for 1:500 dilution).



PRECISION

Intra-assay precision (CV) (n=10)
Sample 1 (17.2 Units): 4.1%

Intra-assay precision (CV) (n=10)
Sample 1 (16.5 Units): 8.6%

SHORT ASSAY PROTOCOL

A. Preparation

1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Dilute samples with diluent sample 1:100
4. Dilute freshly HRP conjugate 1:100 with diluent conjugate

B. Performance

1. Pipette 100 µl of samples, standards, controls into the wells
2. Incubate for 2 hours at 4°C temperature
3. Wash three times with 300 µl of wash buffer
4. Dispense 100 µl of HRP conjugate solution
5. Incubate for 1 hour (with shaking) at room temperature
6. Wash three times with 300 µl of wash buffer
7. Dispense 100 µl of TMB substrate solution
8. Incubate for 20 minutes at room temperature in the dark
9. Add 100 µl of stop solution
10. Measure absorption at 450 nm / 620 nm

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 617-419-2019.