Datasheet for ABIN454544

Mu Opioid Receptor 1 ELISA Kit

Go to Product page

Overview

Quantity:	96 tests
Target:	Mu Opioid Receptor 1 (OPRM1)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.156-10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the specific measurement of Human beta-Endorphin recepter,beta-EPR concentrations in cell culture supernates, serum, and plasma.
Sample Type:	Cell Culture Supernatant, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural Human beta-EPR.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	< 39 pg/mL The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.
Characteristics:	Homo sapiens,Human,Mu-type opioid receptor,M-OR-1,MOR-1,Mu opiate receptor,Mu opioid

Product Details

	receptor,MOP,hMOP,OPRM1,MOR1
Components:	Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1x20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1x120µl), Detection Reagent B (1x120µl), Wash Buffer(25 x concentrate) (1x30ml), Substrate (1x10ml), Stop Solution (1x10ml)

Target Details

Target:	Mu Opioid Receptor 1 (OPRM1)
Alternative Name:	OPRM1 (OPRM1 Products)
Background:	Endorphins are endogenous opioid biochemical compounds. They are polypeptides produced by the pituitary gland and the hypothalamus in vertebrates, and they resemble the opiates in their abilities to produce analgesia and a sense of well-being. In other words, they might work as ",natural pain killers.", Using drugs may increase the effects of the endorphins. The term ",endorphin", implies a pharmacological activity (analogous to the activity of the corticosteroid category of biochemicals) as opposed to a specific chemical formulation. It consists of two parts: endo- and -orphin, these are short forms of the words endogenous and morphine, intended to mean ",a morphine-like substance originating from within the body.", The term endorphin rush has been adopted in popular speech to refer to feelings of exhilaration brought on by pain, danger, or other forms of stress,supposedly due to the influence of endorphins. However, this term does not occur in the medical literature. Beta-endorphin is released into the blood (from the pituitary gland) and into the spinal cord and brain from hypothalamic neurons. The beta-endorphin that is released into the blood cannot enter the brain in large quantities because of the blood-brain barrier. The physiological importance of the beta-endorphin that can be measured in the blood is far from clear: beta-endorphin is a cleavage product of POMC which is the precursor hormone for adrenocorticotrophic hormone (ACTH). The behavioural effects of beta-endorphin are exerted by its actions in the brain and spinal cord, and probably the hypothalamic neurons are the major source of beta-endorphin at these sites. In situations where the level of ACTH is increased, the level of endorphins also increases slightly. Beta-endorphin has the highest affinity for the µ1-opioid receptor, slightly lower affinity for the µ2- and delta-opioid receptors and low affinity for the kappa1-opioid receptors. µ-receptors are the main receptor through which morphine acts. Classically, µ-receptors are presynaptic, and inhibit neurotransmitter release, through this mechanism, they inhibit the release of the inhibitory neurotransmitter GABA, and disinhibit the dopamine pathways, causing more dopamine to be released. By hijacking this process, exogenous opioids cause inappropriate dopamine release, and lead to aberrant synaptic plasticity which causes addiction. Opioid receptors have many

Target Details

	other and more important roles in the brain and periphery however, modulating pain, cardiac, gastric and vascular function as well as possibly panic and satiation, and receptors are often found at 2 postsynaptic locations as well as presynaptically.
Pathways:	cAMP Metabolic Process, Synaptic Membrane

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for beta-EPR has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any beta-EPR present is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for beta-EPR is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of beta-EPR bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (10,000 pg/mL). The Sample Diluent serves as the zero standard (0 pg/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.
Sample Collection:	Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Note: Citrate plasma has not been validated for use in this assay.
Assay Procedure:	Allow all reagents to reach room temperature. Arrange and label required number of strips.

	1. Prepare all reagents, working standards and samples as directed in the previous sections. 2. Add 100 uL of Standard, Control, or sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C. 3. Remove the liquid of each well, don't wash. 4. Add 100 uL of Detection Reagent A to each well. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 6. Add 100 uL of Detection Reagent B to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37 °C. 7. Repeat the aspiration/wash as in step 5. 8. Add 90 uL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light. 9. Add 50 uL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. Important Note: 1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available. 3. Duplication of all standards and specimens, although not required, is recommended. 4. When mixing or reconstituting protein solutions, always avoid foaming. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average

Application Details

	zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the beta-EPR concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the 3 Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.