

PRODUCT DATASHEET
ChemiScreen™ A₃ Adenosine Membrane Preparation

CATALOG NUMBER:	HTS052M	QUANTITY:	200 units
LOT NUMBER:	SC20170814	VOLUME/CONCENTRATION	1 mL, 1 mg/mL

BACKGROUND: Extracellular adenosine mediates a multitude of biological effects, including wakefulness, antiarrhythmia, bronchoconstriction and response to ischemia and oxidative stress. A family of four GPCR adenosine receptors, A₁, A_{2A}, A_{2B} and A₃, is responsible for these effects (Fredholm et al., 2001). A₃, which couples to G_{i/o}, is expressed in mast cells along with A_{2B}. Mice lacking A₃ display reduced mast cell degranulation and bronchoconstriction in response to adenosine (Tilley et al., 2003; Zhong et al., 2003). Eurofins cloned human A₃-expressing cell line is made in the Chem-3 host, which supports high levels of recombinant A₃ expression on the cell surface and contains high levels of the promiscuous G protein G α 15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at A₃.

Eurofin Discovery's A₃ membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of antagonists of A₃ interactions with its ligands. The membrane preparations exhibit a K_d of 0.4 nM for [¹²⁵I]-AB-MECA. With 5 μ g/well A₃ Membrane Prep and 5nM [¹²⁵I]-AB-MECA, a greater than 3-fold signal-to-background ratio is obtained.

APPLICATIONS: Radioligand Binding Assay

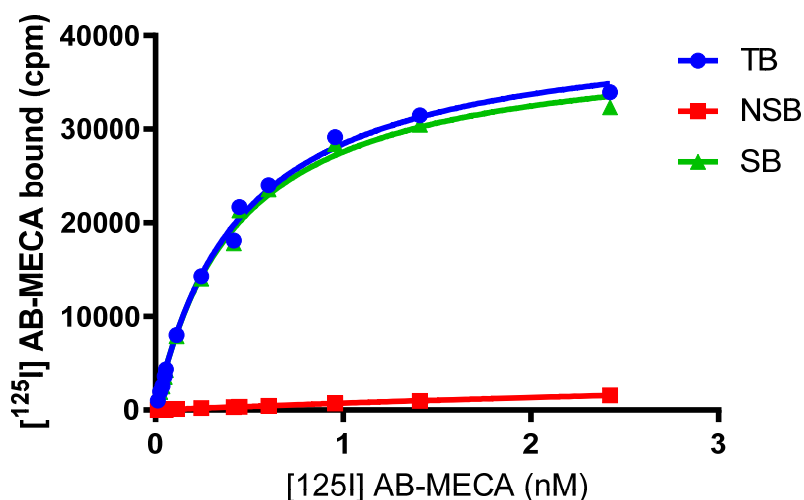


Figure 1. Saturation binding for A₃. 5 μ g/well A₃ Membrane Preparation was incubated with increasing amount of ¹²⁵I-labeled AB-MECA in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled IB-MECA. Specific binding was determined by subtracting NSB from TB.

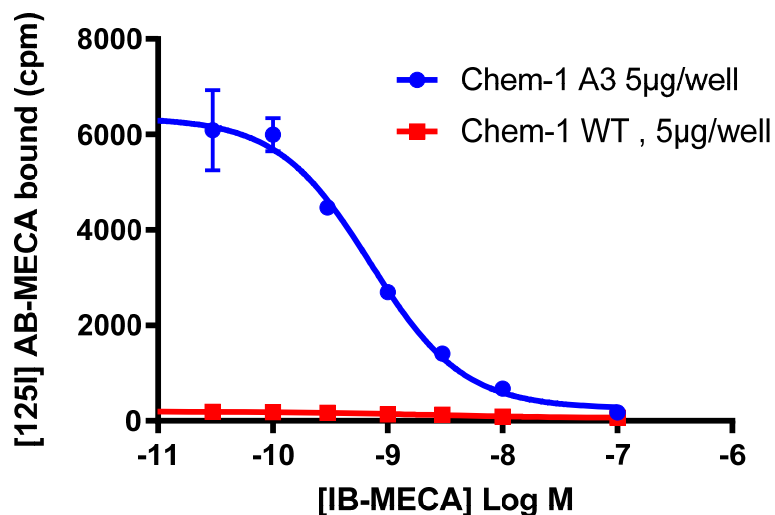


Figure 2. Competition Binding for A₃. A₃ Membrane Preparation (5 µg/well) and wild type Chem-3 Membrane Preparation were incubated with 0.2 nM ¹²⁵I-labeled AB-MECA and increasing concentrations of unlabeled IB-MECA. More than 3-fold signal:background was obtained with A₃ membranes, whereas no binding was observed with wild type membranes.

SPECIFICATIONS: 1 unit = 5 µg
 B_{max}: 2.24 pmol/mg
 K_d: 0.4 nM
 Signal:Background: ≥3-fold

HOST CELLS: Chem-3, a suspension mammalian cell line without any endogenous A₃ expression.

Species: Human A₃ (Accession number L22607)

RECOMMENDED BINDING ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate and incubated for 30 min at room temperature. Prior to filtration, a GF/C 96-well harvest plate was coated with 0.3% polyethyleneimine. The binding reactions were transferred to the filter plate, and washed 7 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted to determine receptor-associated radioligand binding.

Binding Buffer: 50 mM Tris, pH 7.4, 10 mM MgCl₂, 1 mM EDTA, filtered and stored at 4°C. Ligands were diluted in binding buffer containing 10% DMSO, and were then added to membranes such that the final DMSO concentration was 1%.

Radioligand: [¹²⁵I]-AB-MECA (Perkin Elmer# :NEX312)

Wash Buffer: 50 mM Tris, pH 7.4, filtered and stored at 4°C.

One vial contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield approximately a 3-fold signal:background ratio with [³H]-CP55940 at 2 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol, and 1% BSA with no preservatives.

Packaging method: Membrane proteins were adjusted to 1 mg/mL in packaging buffer, dispensed at 1 mL per vial, rapidly frozen, and stored at -80°C.

**STORAGE/
HANDLING:**

Store at -70°C . Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw this product.

REFERENCES:

1. Fredholm, BB et al. (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53: 527-552.
2. Tilley SL et al. (2003) Identification of A3 receptor- and mast cell-dependent and -independent components of adenosine-mediated airway responsiveness in mice. *J. Immunol.* 2003 Jul 1;171(1):331-7
3. Zhong H et al. (2003) Activation of murine lung mast cells by the adenosine A3 receptor. *J. Immunol.* 171: 338-45.

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