eurofins | Discovery Services

# PRODUCT DATASHEET

## ChemiScreen™ C3AR Anaphylatoxin Receptor Membrane Preparation

| | | | |
|---|---|---|---|
| **CATALOG NUMBER:** | HTS016M | **QUANTITY:** | 200 units |
| **LOT NUMBER:** | SC192271 | **VOLUME/CONCENTRATION:** | 1 mL, 2 mg/mL |

**BACKGROUND:**

C3a, along with C4a and C5a, is a 77 amino acid anaphylotoxin generated by proteolytic cleavage during activation of the complement pathway (Ember and Hugli, 1997). The anaphylotoxins strongly promote inflammation by recruiting leukocytes, particularly basophils, eosinophils, neutrophils and monocytes (Martin *et al*., 1997). The proinflammatory properties of C3a are mediated by interaction between the peptide and a 7-TM G-protein coupled receptor, C3aR (Crass *et al*., 1996). Genetic and pharmacological inhibition of C3a/C3aR interaction indicates an important role for C3aR in allergic asthma (Humbles *et al.*, 2000; Drouin *et al.*, 2002). C3a/C3aR also enhances the effect of SDF-1 in promoting retention of haematopoietic stem/progenitor cells within the bone marrow (Rataiczak *et al.*, 2004). The C3aR membrane preps 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 agonists and antagonists of C3aR. The membranes have been shown to bind to $^{125}$I-labeled C3a with a $K_d$ of 0.15 nM. With 0.2 nM [$^{125}$I]-C3a, 10 μg/well C3aR Membrane Prep yields greater than 15 fold signal-to-background ratio.

**APPLICATIONS:**

Radioligand binding assay

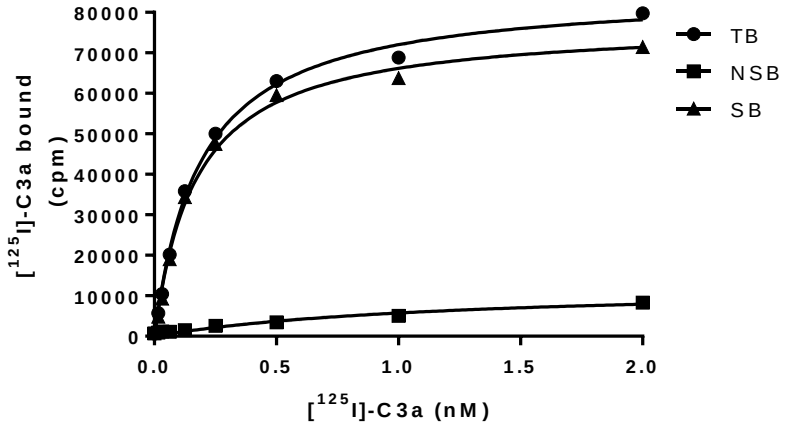


**Figure 1. Saturation binding for C3aR.** 10 ug/well C3aR Membrane Preparation was incubated with increasing amount of [$^{125}$I]-C3a in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled C3a. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from lot.


**Eurofins Pharma Bioanalytics Services US Inc.**

15 Research Park Drive
St Charles MO 63304
USA

T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

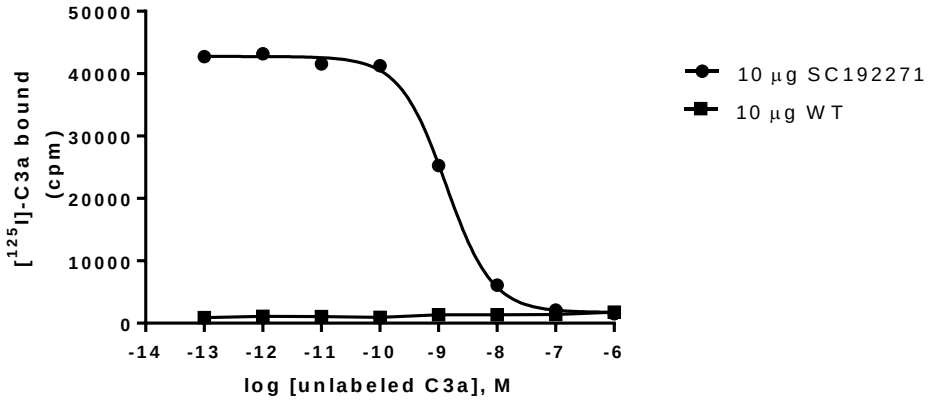


**Figure 2. Competition binding for C3aR.** 10 µg/well C3aR Membrane Preparation or Chem-1 Wild-type Membrane Preparation (WT; EMD Millipore HTS000MC1) was incubated with 0.2 nM [$^{125}$I]-C3a and increasing concentrations of unlabeled C3a. Greater than 15-fold signal:background was obtained with C3aR Membrane Preparation. Sample data from lot SC192271

**SPECIFICATIONS:** 1 unit = 10 µg
Bmax: 3.1 pmol/mg
$K_d$: 0.17 nM

**TRANSFECTION:** Full-length human C3aR cDNA (Accession Number: U28488)

**Species:** Human

**HOST CELLS:** Chem-1, an adherent cell line with no detectable endogenous C3aR expression

**RECOMMENDED ASSAY CONDITIONS:** Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, an FC 96-well harvest plate (EMD Millipore cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.
**Binding buffer:** 50 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C.
**Radioligand:** : [$^{125}$I] C3a (Perkin Elmer # NEX356)

**Wash Buffer:** 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4°C.
One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 5-fold signal:background with $^{125}$I-labeled C3a at 0.2 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 the indicated concentration in 1 ml packaging buffer, 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.

**REFERENCES:**


1. Ember JA and Hugli TE (1997) Complement factors and their receptors. *Immunopharmacology* 38: 3-15
2. Martin U, *et al*. (1997*)* The human C3a receptor is expressed on neutrophils and monocytes, but not on B or T lymphocytes. *J. Exp. Med.* 186: 199-207.
3. Crass T *et al.* (1996) Expression cloning of the human C3a anaphylatoxin receptor (C3aR) from differentiated U-937 cells. *Eur. J. Immunol.* 26: 1944-50.
4. Humbles AA *et al.* (2000) A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 406: 998-1001.
5. Drouin SM *et al.* (2002) Absence of the complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy.*J. Immunol.* 169: 5926-33.
6. Ratajczak J *et al.* (2004) Mobilization studies in mice deficient in either C3 or C3a receptor (C3aR) reveal a novel role for complement in retention of hematopoietic stem/progenitor cells in bone marrow. *Blood* 103: 2071-8.