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PRODUCT DATASHEET

ChemiScreen™ C5aR Anaphylatoxin Membrane Preparation

CATALOG NUMBER:	HTS017M	**QUANTITY:**	200 units
LOT NUMBER:	SC125427	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

C5a is a proinflammatory peptide generated through activation of the complement system, and is more potent than the other anaphylatoxins, C4a and C3a, in activating peripheral blood leukocytes (Gerard and Gerard, 1994). It can actively participate in the regulation of local cytokine network by stimulating production of proinflammatory cytokines (Buchner *et al*., 1995; Hsu *et al*., 1999). The proinflammatory effects of C5a are mediated through binding to a specific 7-TM chemoattractant receptor, C5aR (CD88). Binding of C5a to phagocyte C5aR induces chemotaxis, production of superoxide anions, and release of degradative enzymes. Pharmacologic or genetic disruption of C5a/C5aR interaction reduces sepsis (Riedemann *et al.*, 2003), immune complex-induced lung disease (Shushakova *et al.*, 2002), and Arthrogen-induced arthritis (Grant *et al.*, 2002) in experimental models. Cloned human C5a receptor membrane preparations are ideal tools for screening for antagonists of C5a/C5aR interactions. The membrane preparations exhibit a Kd of 0.45 nM for [^{125}I]-C5a, complement. With 0.1 nM [^{125}I]-C5a, 5 µg/well of C5aR Membrane Prep yields greater than a 5-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

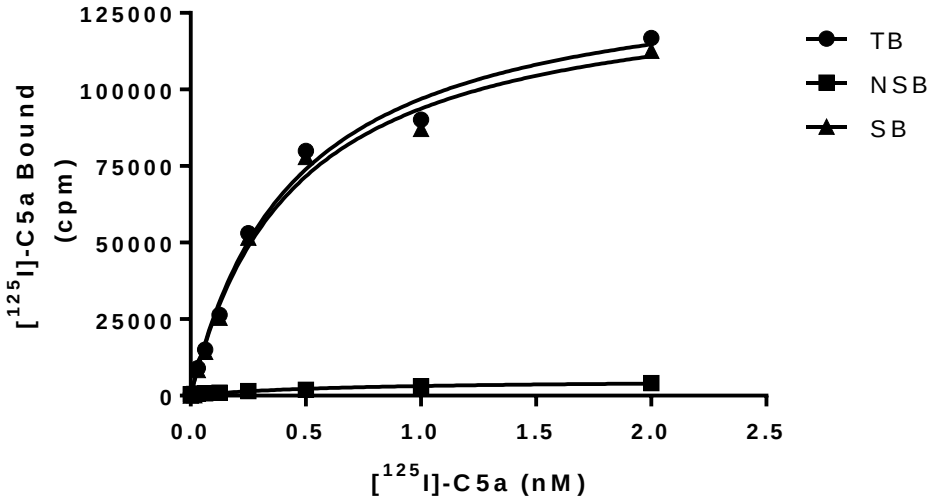

Figure 1. Saturation Binding for C5aR. 5 µg/well of C5aR Membrane Preparation was incubated with [^{125}I]-C5a in the absence (TB = total binding) or presence (NSB = nonspecific binding) of a 200-fold excess of unlabeled C5a. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample from lot SC125427.

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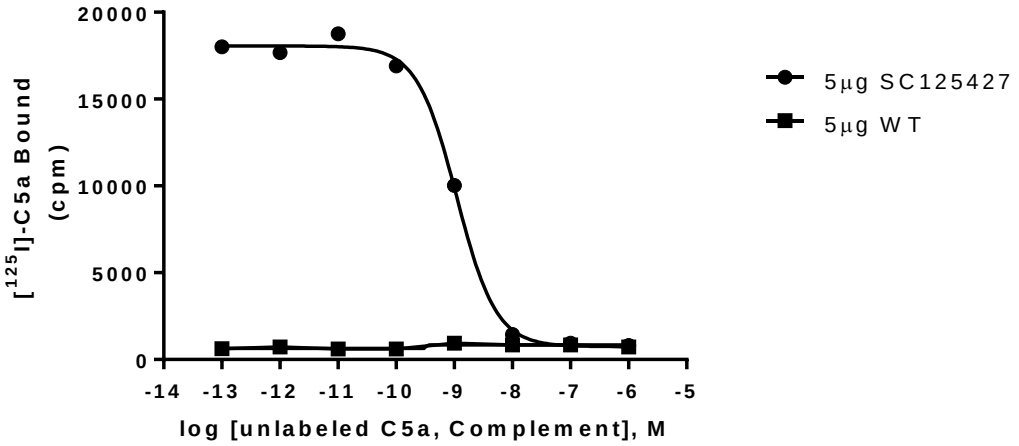

Figure 2. Competitive Binding for C5aR. C5aR Membrane Preparation (5 µg/well in a 96-well plate) was incubated with 0.1 nM [^{125}I]-C5a and increasing concentrations of unlabeled C5a, and subjected to filtration binding. The data are from a representative sample from lot SC125427.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]-C5a Binding: 11.1 pmol/mg protein
K_d for [^{125}I]-C5a Binding: 0.45 nM
Signal:background: >5-fold

TRANSFECTION: Full-length human C5aR cDNA (Accession Number: M62505)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous expression of C5a receptor.

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 non-binding 96-well plate, and incubated for 2 h. Prior to filtration, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then 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]-C5a (PerkinElmer # NEX250)

Wash Buffer: 50 mM HEPES, pH 7.4, 500 mM 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]-C5a at 0.1 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Buchner RR *et al*. (1995). Expression of functional receptors for human C5a anaphylatoxin (CD88) on the human hepatocellular carcinoma cell line HepG2. Stimulation of acute-phase protein-specific mRNA and protein synthesis by human C5a anaphylatoxin.*J. Immunol*. 155:308.

2. Gerard C and Gerard NP (1994). C5A anaphylatoxin and its seven transmembrane-segment receptor. *Annu.Rev.Immunol*. 12:775-808.

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4. Hsu MH *et al.* (1999). NF-kappaB activation is required for C5a-induced interleukin-8 gene expression in mononuclear cells. *Blood* 93:3241-3249.

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