MILLIPORE

CHEMICON
now part of Millipore

CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN RECOMBINANT APJ APELIN RECEPTOR

CATALOG NUMBER:	HTS068M	**QUANTITY:**	200 units
LOT NUMBER:	R0711E0015	**VOLUME/CONCENTRATION PER VIAL:**	1 mL, 1 mg/mL

BACKGROUND:

Apelin peptides have been discovered to be a family of peptides of different sizes that is derived from the N-terminus of a 77 amino acid precursor peptide (preproapelin) (Hosoya *et al.*, 2000). Apelin receptor (APJ) is a G protein-coupled receptor that is activated by several apelin fragments. APJ activation leads to the inhibition of cAMP production. APJ and apelin peptides have been found to be involved in the regulation of cardiovascular function (Katugampola *et al.*, 2001) and fluid homeostasis (Reaux *et al.*, 2001). Broad roles of apelin system has been established in lowering blood pressure, as a potent cardiac inotrope, in modulating pituitary hormone release and food and water intake, in stress activation, and as a novel adipokine that is excreted from fat cells and regulates insulin (Lee *et al.*, 2006) . Millipore's APJ 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 agonists and antagonists of APJ. The membrane preparations exhibit a Kd of 0.4 nM for [^{125}I]-Apelin. With 0.25 nM [^{125}I]-Apelin, 5 µg/well APJ Membrane Prep typically yields greater than 20-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay and GTPγS binding.

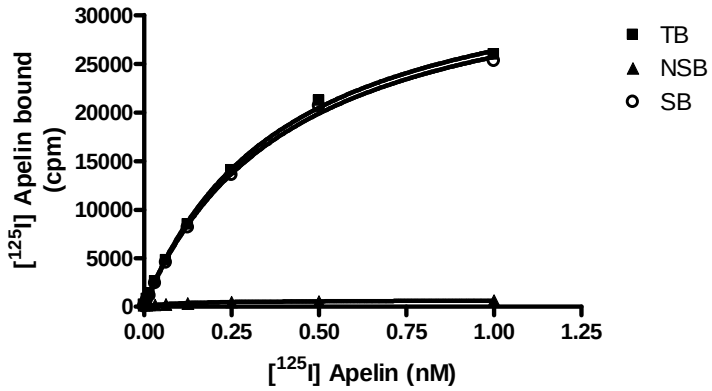

Figure 1. Saturation binding for APJ. 5 µg/well APJ Membrane Preparation was incubated with increasing amount of ^{125}I-labeled Apelin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled apelin-13. Specific binding (SB) was determined by subtracting NSB from TB.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com

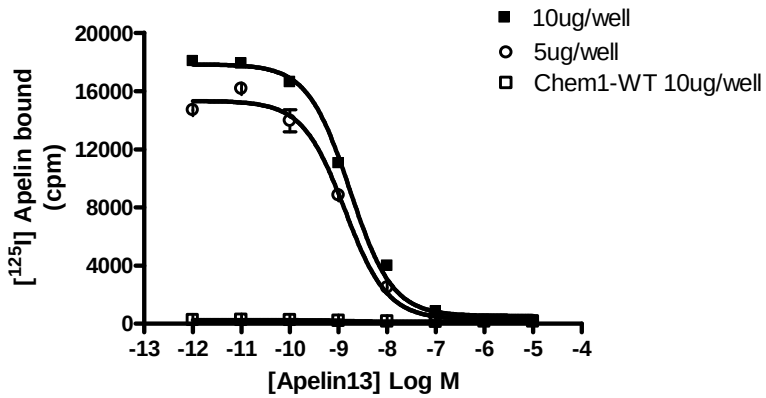

Figure 2. Competition binding for APJ. 10 and 5µg/well APJ Membrane Preparation and wild-type Chem-1 Membrane Preparation (Chemicon catalog # HTS000MC1) were incubated in a 96-well plate with 0.25 nM ^{125}I-labeled apelin and increasing concentrations of unlabeled apelin-13. More than 20-fold signal:background was obtained

Table 1. Signal:background and specific binding values obtained in a competition binding assay with varying amounts of APJ membrane prep.

	10 µg/well	5 µg/well
Signal:background	33.0	49.7
Specific binding (cpm)	17297	15015

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]-Apelin binding: 3.85 pmol/mg protein
K_d for [^{125}I]-Apelin binding: ~0.4 nM

TRANSFECTION: Full-length human AGTRL1 cDNA encoding APJ (Accession Number: U03642)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous apelin 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 (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℃

Radioligand: [^{125}I]-Apelin. (Perkin Elmer#:NEX-393)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4℃ .

One package contains enough membranes for at least 200 assays (units), where a unit is

MILLIPORE

CHEMICON
now part of Millipore

the amount of membrane that will yield greater than 20-fold signal:background with ^{125}I labeled Apelin at 0.25 nM

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membranes protein were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Maintain frozen at –70°C for up to 2 years. Do not freeze and thaw.

REFERENCES:

Hosoya M *et al.* (2000) Molecular and functional characteristics of APJ: Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J. Biol. Chem.* 275: 21061–21067.

Katugampola SD *et al.* (2001) [(125)I]-(Pyr(1))Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *Br. J. Pharmacol.* 132: 1255-1260.

Reaux A *et al.* (2001) Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. *J. Neurochem.* 77: 1085-1096.

Lee DK *et al.* (2006) Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. *Trends Pharmacol. Sci.* 27: 190-194.

For research use only; not for use as a diagnostic.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com