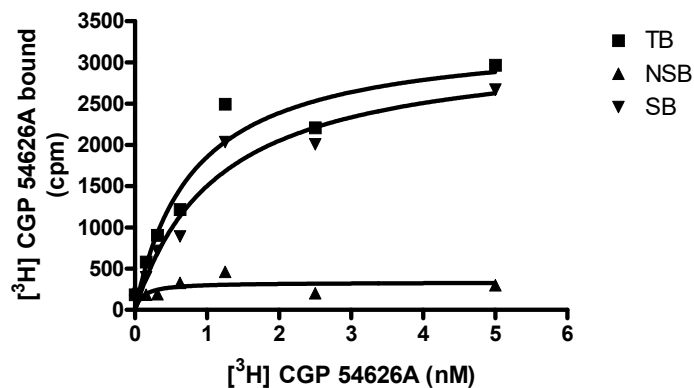


**PRODUCT DATASHEET**
**ChemiScreen™ GABA<sub>B1b</sub>/GABA<sub>B2</sub> Serotonin Membrane Preparation**

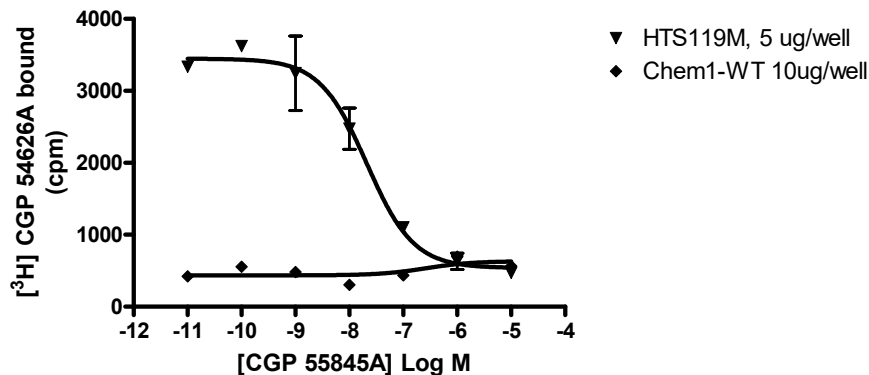
<b>CATALOG NUMBER:</b>	HTS119M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>	2150335	<b>VOLUME/CONCENTRATION:</b>	1 mL, 1 mg/mL

**BACKGROUND:** The neurotransmitter  $\gamma$ -aminobutyric acid (GABA) exerts its effects through an ion channel, GABA<sub>A</sub>, and a GPCR, GABA<sub>B</sub>. Functional GABA<sub>B</sub> is a heterodimer composed of the GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits, which share 35% sequence identity and belong to the class 3 family of GPCRs. The GABA<sub>B1</sub> subunit, which exists as splice variants GABA<sub>B1a</sub> and GABA<sub>B1b</sub>, binds directly to GABA and is required for agonist activation. The GABA<sub>B2</sub> and GABA<sub>B1</sub> subunits associate by formation of a coiled coil by their C-terminal tails; this association masks an ER retention sequence in GABA<sub>B1</sub> to permit export from the ER and trafficking to the cell surface. In addition to its chaperone function, GABA<sub>B2</sub> is the component that couples to G<sub>i</sub> to reduce intracellular cAMP. Agonists of GABA<sub>B</sub>, such as baclofen, are used clinically for treatment of muscle spasticity, migraine headache and musculoskeletal pain (Bowery *et al.*, 2002). GABA<sub>B1b</sub> 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 GABA<sub>B1b</sub> receptor interactions with its ligand. The membrane preparations exhibit a K<sub>d</sub> of 1.15nM for [<sup>3</sup>H]-CGP 54626A. With 5.0 ug/well GABA<sub>B1b</sub> Membrane Prep and 7.5nM [<sup>3</sup>H]-CGP 54626A, a greater than 5-fold signal-to-background ratio was obtained

**APPLICATIONS:** Radioligand binding assay



**Figure 1. Saturation binding for GABA<sub>B1b</sub> Receptor.** 5.0  $\mu$ g/well GABA<sub>B1b</sub> Membrane Preparation was incubated with increasing amount of [<sup>3</sup>H]-CGP 54626A in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled human recombinant GABA<sub>B1b</sub> receptor. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.



**Figure 2. Competition binding for GABA<sub>B1b</sub> Receptor.** GABA<sub>B1b</sub> Receptor Membrane Preparation (5mg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 7.5nM [<sup>3</sup>H]-CGP 54626A and increasing concentrations of unlabeled CGP 55845A, and more than 5- fold signal:background was obtained. Representative sample data.

**SPECIFICATIONS:** 1 unit = 5 µg membrane preparation  
 B<sub>max</sub>: 8.49 pmol/mg protein  
 K<sub>d</sub>: 1.15 nM  
 Signal:background: >5

**TRANSFECTION:** Full-length human GABBR1b and GPR51 cDNAs encoding GABA<sub>B1b</sub>/GABA<sub>B2</sub> (Accession Numbers: NM\_021903 and NM\_005458)

**Species:** Human

**HOST CELLS:** Chem-1, an adherent mammalian cell line without any endogenous 5-HT<sub>2c</sub> 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. 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.

**Assay Buffer:** 1X Hanks' Balanced Salt Solution (HBSS): GIBCO Cat. No. 14175

**Radioligand:** [<sup>3</sup>H]-CGP 54626A (American Radiolabeled Chemicals Inc., ART 517)

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 5-fold signal:background with <sup>3</sup>H-labeled CGP 54626A at 7.5nM.

**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. Bowery NG *et al.* (2002) International Union of Pharmacology. XXXIII. Mammalian  $\gamma$ -aminobutyric acid<sub>B</sub> receptors: Structure and function. *Pharmacol. Rev.* 54: 247-264.

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