

**CLOINED 5HT1A SEROTONIN RECEPTOR, HUMAN
EXPRESSED IN CHO CELLS, COATED ON FLASHPLATE®**

Catalog No.: **6120501**

Date of manufacture:

Lot No.: **Typical**

Storage: **Store at 4°C in the dark.**

Stability: This product is stable for **three months** when stored in its original packaging according to the recommended storage conditions.

IDENTIFICATION**RECEPTOR**

Name: **5HT1A Serotonin**

Species: **Human**

GenBank access #: **X13556**

Expression System: **CHO cells**

Type of FlashPlate: **Basic FlashPlate (NEN Cat. No. SMP200)**

Number of amino acids: **421**

Polypeptide predicted M.W. (Da): **46029**

Mass membrane protein/well: **10 µg**

Receptors/well: **8.7 fmoles**

RADIOLIGAND BINDING ASSAY PROTOCOLS

Recommended Binding Buffer: 50 mM TRIS-HCl pH 6.5
10 mM MgCl₂
0.5 mM EDTA
0.1% Ascorbic acid
0.5% BSA

Recommended Assay Volume: 25 µl

FlashPlate preparation: Let the FlashPlate equilibrate at room temperature for one hour;
Remove TopSeal-A from FlashPlate just before starting the assay.

Assay validation:**A) Saturation:** (use 48 wells: A1 to H6)

- Prepare the radioligand¹ at 2X final concentration (2 X 6 nM = 12 nM) in binding buffer;
- Using this 2X radioligand stock, dilute 7 times using serial doubling dilutions;
- Prepare the unlabeled ligand² at 2X final concentration (2 X 10 µM = 20 µM) in binding buffer;
- Add 12.5 µl of buffer to 24 wells (A1-A3, B1-B3,...H1-H3) for total binding determination;
- Add 12.5 µl of unlabeled ligand to 24 different wells (A4-A6,B4-B6,...H4-H6) for non-specific binding determination;
- Add 12.5 µl of radioligand from each of the 8 different dilutions, using 6 wells per concentration i.e. 3 wells for total binding and 3 wells for non-specific binding.



RADIOLIGAND BINDING ASSAY PROTOCOLS (continued)**B) Competition:** (use 48 wells: A7 to H12)

- Prepare the radioligand¹ at 2X final concentration (2 X 0.55 nM = 1.1 nM) in binding buffer;
- Prepare the unlabeled ligand² at 2X final concentration (2 X 3 μM = 6 μM) in binding buffer;
- Using this 2X stock of unlabeled ligand, dilute 14 times using serial doubling dilutions;
- Add 12.5 μl from each of the 15 different dilutions of unlabeled ligand, using 3 wells per concentration (A7-A9, A10-A12, ... G10-G12)
- Add 12.5 μl of buffer to 3 different wells for total binding determination (H10-H12);
- Add 12.5 μl of radioligand to all 48 wells.

C) S/B ratio and intraplate CV determination: (use 96 wells)

- Prepare the radioligand¹ at 2X final concentration (2 X 0.55 nM = 1.1 nM) in binding buffer;
- Prepare the unlabeled ligand² for non-specific binding determination at 2X final concentration (2 X 10 μM = 20 μM) in binding buffer;
- Add 12.5 μl of buffer to 48 wells (A1-A12, B1-B12, ... D1-D12) for total binding determination;
- Add 12.5 μl of unlabeled ligand to 48 different wells (E1-E12, F1-F12, ... H1-H12) for non-specific binding determination;
- Add 12.5 μl of radioligand to all 96 wells.

HTS assay (use 96 wells)

- Prepare the radioligand¹ at 5X final concentration (5 X 0.55 nM = 2.75 nM) in binding buffer;
- Prepare the unlabeled ligand² for non-specific binding determination at 50X final concentration (50 X 10 μM = 0.5 mM) in binding buffer (positive control);
- Prepare the compounds to be tested at 50X final concentration in 100% DMSO;
- Add 19.5 μl of binding buffer to all 96 wells;
- Add 0.5 μl of 100% DMSO (2% DMSO final concentration) to 5 wells (A1-E1) for total binding determination AND;
- Add 0.5 μl of unlabeled ligand to 3 wells (F1-H1) for non-specific binding determination;
- Add 0.5 μl of compounds to be tested to 88 remaining wells (A2-H12);
- Add 5 μl of radioligand to all 96 wells.

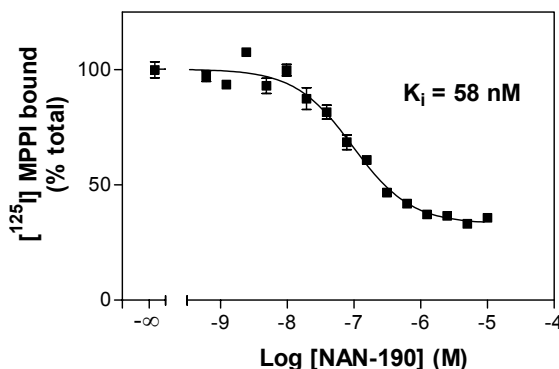
Detection protocol

- Apply TopSeal-A (NEN Cat. No.: SMP201) onto FlashPlate microplates;
- Incubate at room temperature for 60 minutes;
- Count FlashPlate on TopCount™ Microplate Scintillation and Luminescence Counter³.

¹ BioSignal use [¹²⁵I] MPPI (NEN Catalog No: NEX-336) as radioligand.

² BioSignal recommends using NAN-190 as unlabeled ligand.

³ BioSignal recommends a count delay of one (1) minute prior to counting and a counting time of 30 seconds per well.

COMPETITION BINDING CURVE

Competition binding assay was performed using human h5HT_{1A} Serotonin ScreenReadyTarget product (lot number: 501-65001) according to protocol B. The values represent the mean ± SEM from triplicate wells. Counts in absence of ligand (100%) were 4959 ± 172 CPM, counts at maximal ligand concentration were 1773 ± 64 CPM.

QUALITY CONTROL

Signal to Background Ratio (S/B)¹: **3.4** Coefficient of Variation (CV)²: **6.8%**

Screening window coefficient (Z')³: **0.62**

B_{max}⁴: **0.87 pmole/mg**

[¹²⁵I] MPPI K_d value⁴: **2.17 nM**

Reference compound: **NAN-190**

Reference compound K_i⁵: **58 nM**

Solvent compatibility: In the course of product development, ligand binding on this receptor was not affected in the presence of 5% DMSO, 1% Dimethyl formamide, 5% Methanol or 0.1% Acetonitrile. BioSignal recommends performing a solvent compatibility experiment since lot to lot variation may occur.

¹ S/B is defined as the mean of total binding (n=48) divided by the mean of non-specific binding (n=48) using protocol C described above.

² CV is defined as the standard deviation of total binding (n=48) divided by the mean of total binding and multiplied by 100 using protocol C described above.

³ Z' is the screening window coefficient, defined as the ratio of the separate band to the signal dynamic range of the assay using protocol C described above (Zhang *et al.* (1999) J.Biomol.Screen. 2:67-73).

⁴ B_{max} and K_d were determined from a saturation experiment performed using varying concentrations of [¹²⁵I] MPPI (protocol A). An arbitrary counting efficiency of 50% was considered to obtain the B_{max} value.

⁵ K_i is determined from a competition experiment performed using varying concentrations of NAN-190 (protocol B).

IMPORTANT NOTES

ScreenReadyTargets are sensitive to moisture. Prolonged exposure to ambient air or excessive moisture (e.g. cold room) in the absence of TopSeal A or when removed from the original package may seriously affect assay performance and greatly reduce the shelf life. Precautions should therefore be taken to avoid moisture condensation on the surface of the microplate.

Occasional "spikes" can be observed when counting ScreenReadyTargets. Spikes are generated by static electricity buildup on the plate. The problem can be easily remedied by simply wiping the bottom of the microplate with a damp tissue prior to counting, or by simply recounting the microplate.

Incubation of ScreenReadyTargets under sources of bright light will cause photoluminescence, which will translate into increased background counts. Although absolute count rates are typically low (<100 CPM), photoluminescence will affect non-specific binding counts which will translate into a decrease in S/B ratio values. This "plate glow" effect can become important, especially when using [³⁵S] or [³H] ligands. Performing the incubation under dim light or covering the microplate with an "empty plate" resolves this problem.

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