

**CLONED AT2 ANGIOTENSIN II RECEPTOR, HUMAN,  
EXPRESSED IN HeLa CELLS, COATED ON FLASHPLATE®**Catalog No.: **6120538**

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: **AT2 Angiotensin II**Species: **Human**GenBank access #: **L34579**Expression System: **HeLa cells**Type of FlashPlate: **Basic FlashPlate (NEN Cat. No. SMP200)**Number of amino acids: **363**Polypeptide predicted M.W. (Da): **41200**Mass membrane protein/well: **5 µg**Receptors/well: **2.75 fmoles****RECOMMENDED ASSAY PROTOCOL**

**Recommended Binding Buffer:** 50 mM TRIS-HCl pH 7.4  
5 mM MgCl<sub>2</sub>  
1 mM EDTA  
0.3% BSA

**Recommended Assay Volume:** 25 µl

**FlashPlate preparation:** Let the FlashPlate equilibrate at room temperature for one hour; Remove ScreenReady Target from their original packaging just before starting the assay.

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

- Prepare the radioligand<sup>1</sup> at 2X final concentration (2 X 1.5 nM = 3 nM) in binding buffer;
- Using this 2X radioligand stock, dilute 7 times using serial doubling dilutions;
- Prepare the unlabeled ligand<sup>2</sup> at 2X final concentration (2 X 20 µM = 40 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 radioligand<sup>1</sup> at 2X final concentration (2 X 0.11 nM = 0.22 nM) in binding buffer;
- Prepare the unlabeled ligand<sup>2</sup> at 2X final concentration (2 X 0.3 μM = 0.6 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 radioligand<sup>1</sup> at 2X final concentration (2 X 0.11 nM = 0.22 nM) in binding buffer;
- Prepare the unlabeled ligand<sup>2</sup> for non-specific binding determination at 2X final concentration (2 X 20 μM = 40 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 radioligand<sup>1</sup> at 5X final concentration (5 X 0.11 nM = 0.55 nM) in binding buffer;
- Prepare the unlabeled ligand<sup>2</sup> for non-specific binding determination at 50X final concentration (50 X 20 μM = 1 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.

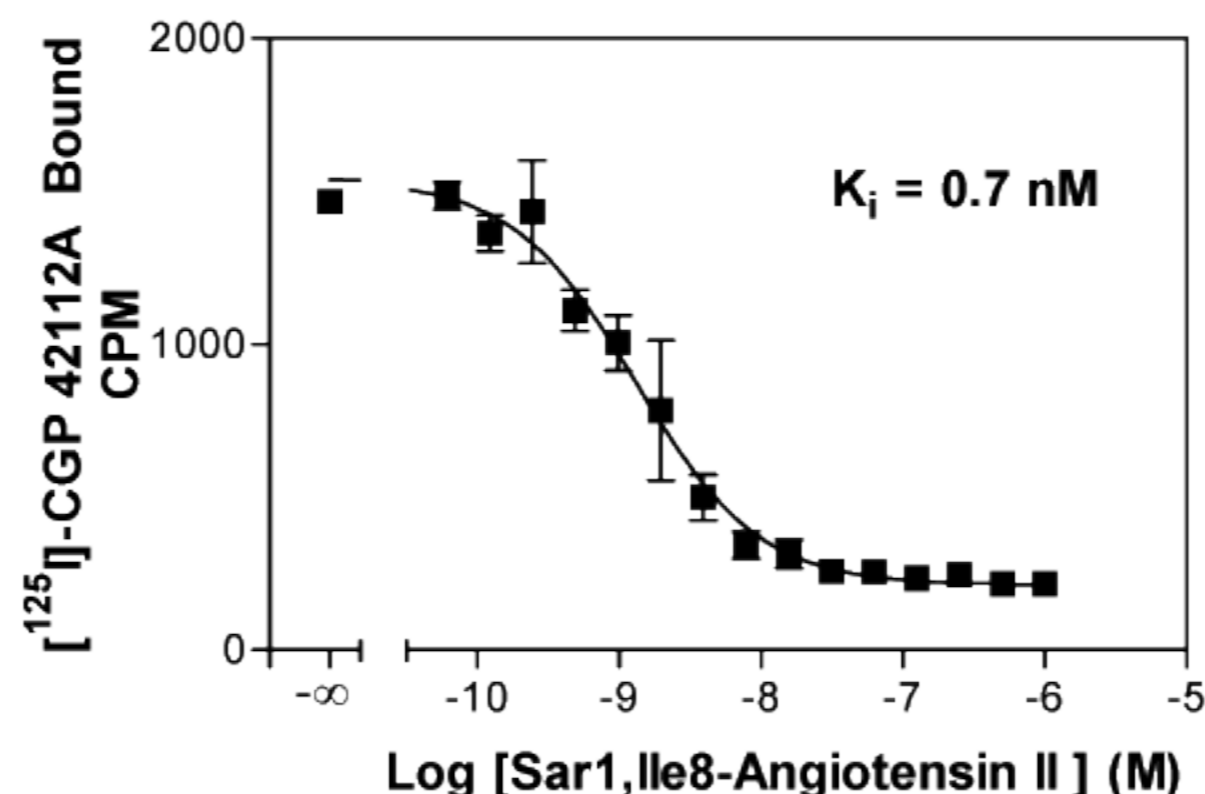
**Detection protocol**

- Apply TopSeal-A (Packard Cat. No.: 6005185) onto FlashPlate microplates;
- Incubate at room temperature for 240 minutes;
- Count FlashPlate on TopCount™ Microplate Scintillation and Luminescence Counter<sup>3</sup>.

<sup>1</sup> BioSignal use [<sup>125</sup>I] CGP 42112A (NEN Catalog No.: NEX-324) as radioligand.

<sup>2</sup> BioSignal recommends using Sar1,Ile8-Angiotensin II as unlabeled ligand.

<sup>3</sup> 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 hAT2 Angiotensin II ScreenReadyTarget product (lot number: 538-65001) according to the protocol B. The values represent the mean ± SEM from triplicate wells.

**QUALITY CONTROL**

Signal to Background Ratio (S/B)<sup>1</sup>: **7.3**      Coefficient of Variation (CV)<sup>2</sup>: **7.0 %**

Screening window coefficient (Z')<sup>3</sup>: **0.70**

B<sub>max</sub><sup>4</sup>: **0.55 pmole/mg**      [<sup>125</sup>I] CGP 42112A K<sub>d</sub> value<sup>4</sup>: **0.24 nM**

Reference compound: **Sar1,Ile8 Angiotensin II**      Reference compound K<sub>i</sub><sup>5</sup>: **0.7 nM**

**Solvent compatibility:** In the course of product development, ligand binding on this receptor was not affected in the presence of 10% DMSO, 1% Dimethyl formamide, 10% Methanol or 5% Acetonitrile. BioSignal recommends performing a solvent compatibility experiment since lot to lot variation may occur.

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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).

<sup>4</sup> B<sub>max</sub> and K<sub>d</sub> were determined from a saturation experiment performed using varying concentrations of [<sup>125</sup>I] CGP 42112A (protocol A). An arbitrary counting efficiency of 50% was considered to obtain the B<sub>max</sub> value.

<sup>5</sup> K<sub>i</sub> is determined from a competition experiment performed using varying concentrations of Sar1,Ile8 Angiotensin II (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 [<sup>35</sup>S] or [<sup>3</sup>H] ligands. Performing the incubation under dim light or covering the microplate with an "empty plate" resolves this problem.

\*\*\*

ScreenReady™Targets and StopSolution™ are trademarks of BioSignal, Inc.

TopCount™ is trademark of Packard Instrument Company

FlashPlate® is a registered trademark of Packard Instrument Company

NEN™ is a trademark of NEN Life Science.

**LIMITED WARRANTY** BioSignal Inc. warrants that, at the time of shipment, the products sold by it are free from defects in material and workmanship and conform to specifications which accompany the product. BioSignal Inc. makes no other warranty, express or implied with respect to the products, including any warranty of merchantability or fitness for any particular purpose. Notification of any breach of warranty must be made within 60 days of receipt unless provided in writing by BioSignal Inc. No claim shall be honored if the customer fails to notify BioSignal Inc. within the period specified. The sole and exclusive remedy of the customer for any liability of BioSignal Inc. of any kind including liability based upon warranty (express or implied whether contained herein or elsewhere), strict liability contract or otherwise is limited to the replacement of the goods or the refunds of the invoice price of goods. BioSignal Inc. shall not in any case be liable for special, incidental or consequential damages of any kind.

**FOR RESEARCH USE ONLY**