

Neurotransmitter Transporter Uptake Assay Kit

A live-cell kinetic assay to measure uptake of serotonin, norepinephrine, and dopamine neurotransmitters

KEY FEATURES

- **Homogeneous, live-cell fluorescent assay**
- **Convenient, single reagent addition assay format**
- **No use of radioactivity required**
- **Amenable to automation and HTS**
- **Analyze on any bottom-read fluorescent microplate instrument**
- **Read in endpoint mode for high-throughput applications**
- **Read in real-time kinetic mode for mechanistic studies**

Introduction

Neurotransmitter transporters have become important pharmaceutical targets, as they play a key role in depression and neurodegenerative diseases (NDD) such as Alzheimer's and Parkinson's diseases. The ability to monitor serotonin, norepinephrine and dopamine neurotransmitter uptake is key to a better understanding of these diseases. Until now, radioactively labeled compounds were used to measure serotonin, norepinephrine and dopamine transporter uptake. With the introduction of this novel assay kit, researchers will now have a tool to screen for live-cell kinetic uptake for these three key neurotransmitters in a homogeneous, fluorescence-based, high-throughput format. The assay can be performed either as a real-time kinetic assay suitable for mechanistic studies or as an HTS-amenable endpoint read.

Homogeneous, simple, and flexible

The homogeneous Neurotransmitter Transporter Uptake Assay has a flexible and simple mix-and-read protocol that can be performed in 96- or 384-well microplates for detection with either kinetic or endpoint modes. It is ideal for use in research, assay development and high-throughput screening applications. This assay improves on current transport and binding assays for these three neurotransmitter transporters, which use radiolabeled ligands, require expensive, specialized equipment, and only detect endpoints. With this assay, researchers now have the ability to measure live-cell kinetics of these important transporters without reliance upon radioactivity and without being limited to endpoint read-only assays.

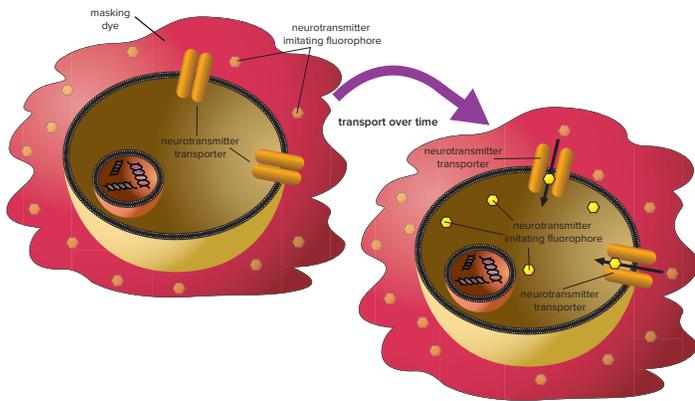


Figure 1. The Neurotransmitter Transporter Uptake Assay principle.

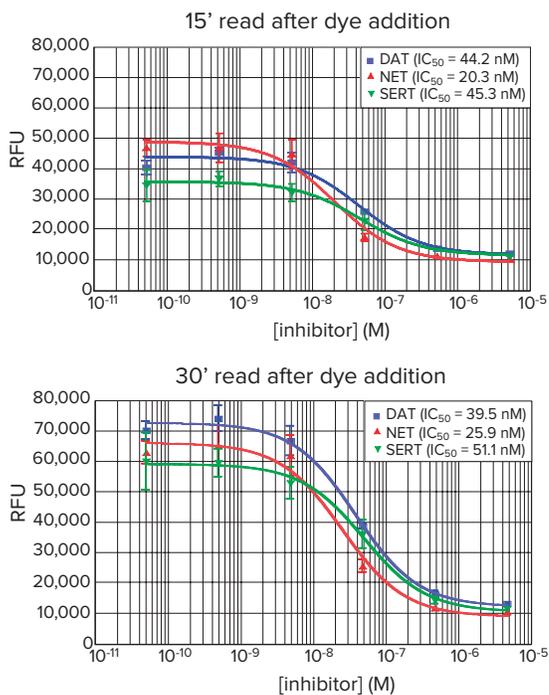


Figure 2. IC₅₀ values comparable at 15' and 30'. IC₅₀ values do not shift if plate read at 15' vs. 30', however, there is a larger assay window when read at 30'.

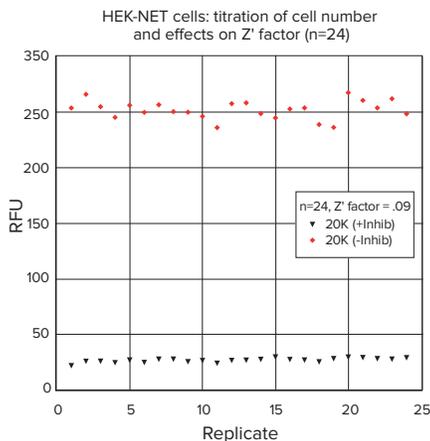


Figure 3. Robust screening assay. Z' factor calculation for NET using no inhibitor as a positive control and maximum inhibitor of 3 μ M Nomifensine as a negative control. With a Z' of 0.9, the assay is shown to be very robust and suitable for HTS applications.

Patented technology

This assay, like all Molecular Devices cell-based assays, utilizes a Molecular Devices masking dye to extinguish extracellular fluorescence. This masking dye technology has been exclusively licensed from Bayer AG (U.S. Patent Nos. 6,420,183, 7,063,952, 7,138,280 and European Patent No. 0,906,572). The assay also includes a novel proprietary fluorescent indicator dye that mimics the neurotransmitters serotonin, norepinephrine, and dopamine, and is actively transported into the cells via the neurotransmitter transporters.

Assay principle

The homogeneous Neurotransmitter Transporter Uptake Assay Kit provides a homogeneous, fast, simple and reliable fluorescence-based assay for the detection of dopamine, norepinephrine or serotonin transporter activity in cells expressing these transporters. The kit employs a fluorescent substrate that mimics the biogenic amine neurotransmitters that is taken into the cell through those specific transporters, resulting in increased intracellular fluorescence intensity (Figure 1). In this convenient no-wash procedure, cells are incubated with the kit reagent and transferred to the plate reader for evaluation. The assay can be performed on any bottom-read mode fluorescence microplate reader and is ideal for use in academic, biotech, and pharmaceutical environments for research, assay development, and screening.

Rapid assay development

The Neurotransmitter Transporter Uptake Assay Kit, with its homogeneous assay protocol, makes this assay very straightforward. Moreover, the concentrations of the novel neurotransmitter indicator and masking dyes and the assay volumes recommended are the result of a very thorough optimization process. Adherence to these recommendations results in maximum assay performance with minimum assay development time. The FlexStation®, SpectraMax® i3/i3x, Paradigm® and M5 Systems are optimal instruments to run this assay for rapid detection and analysis.

This homogeneous, fluorescent assay is robust, sensitive, specific, and can be run in either a kinetic or an endpoint mode. Figure 2 demonstrates the flexibility of the assay as the IC₅₀ values did not notably shift when reading the plate at a later time interval from the first read (at 15 minutes). However, the longer incubation with the dye reagent provided a larger assay window. Figure 3 shows an example of the robust nature of the assay with Z' factor of 0.9 when used with the norepinephrine transporter. Figure 4 illustrates good correlation between the IC₅₀ values with this assay versus expected literature values.

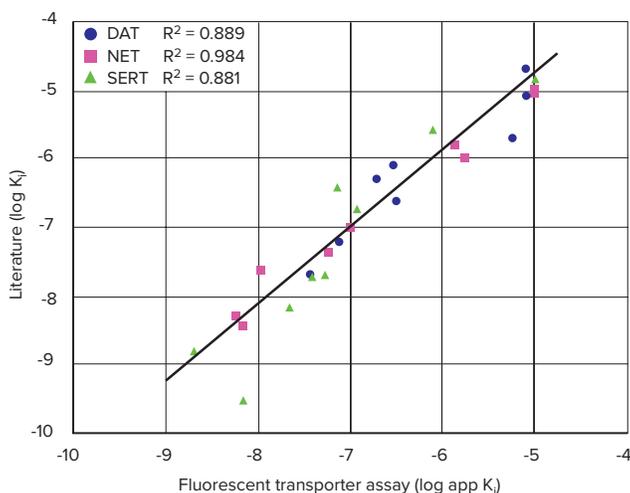


Figure 4. Correlation of IC_{50} values for fluorescent assay vs. published values. Scatter plot correlating apparent K_i determined with the neurotransmitter transporter assay with literature values for known inhibitors of dopamine (DAT), serotonin (SERT), and norepinephrine (NET) re-uptake. The combined correlation coefficient R^2 is greater than 0.9 indicating good correlation between literature and experimental values for this kit.

Product	Description	Part Number
Neurotransmitter Transporter Uptake Assay Evaluation Kit	(2) vials of lyophilized fluorescent dye/masking dye mix. Each vial is sufficient for one (1) 96-well or 384-well plate. Each kit is sufficient for 2 plates total.	R6138
Neurotransmitter Transporter Uptake Assay Explorer Kit	(5) vials of lyophilized fluorescent dye/masking dye mix. Each vial is sufficient for one (1) 96-well or 384-well plate. Each kit is sufficient for 5 plates total.	R8173
Neurotransmitter Transporter Uptake Assay Bulk Kit	(5) vials of lyophilized fluorescent dye/masking dye mix. Each vial is sufficient for ten (10) 96-well or 384-well plates. Each kit is sufficient for 50 plates total.	R8174

Assay performance considerations

Solvent tolerance

This assay can tolerate a final solvent concentration of up to 1% DMSO or Ethanol with minimal impact on the precision or the overall assay performance in the cell lines tested.

Cell density

Density of the cells and cell plating could influence the assay performance as well as the signal readout. Therefore, it is recommended that the cell plating information supplied in the Product Insert be used as a guideline and that further optimization be performed to maximize assay performance.

Same-day cell plating

Cells can be plated on the same day that the assay is run without compromising the assay performance or precision. However, it is advised that the cell number and the amount of time for the cells to adhere to the plate be optimized for the specific cell line.

BSA addition to HBSS buffer

Similar performance is seen with or without the addition of BSA. However, the use of BSA can help reduce binding of compounds to the walls of the plate well or pipette tips.

Order of reagent additions

Changing the order of reagent additions, i.e., dye before inhibitor, may result in a reduced signal. Therefore, following the suggested protocol and guidelines is recommended to ensure the desired assay performance.

Compatible with these Molecular Devices systems



FLIPR® Tetra System



SpectraMax® i3/i3x Multi-Mode Microplate Readers



SpectraMax® Paradigm® Multi-Mode Microplate Reader



FlexStation® 3 Multi-Mode Microplate Reader



SpectraMax® M Series Multi-Mode Microplate Readers



Gemini™ XPS Microplate Reader

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