

R&D, Administration and Europe Office

Cisbio Bioassays
 Phone : +33 (0)4 66 79 67 05
 Fax : +33 (0)4 66 79 19 20
 E-mail : bioassays@cisbio.com

Japan Office

Sctei Medical Labo K.K.
 Phone : +81-3-5510-2932
 Fax : +81-3-5510-0130
 E-mail: reagent@sctimedilabo.co.jp

USA Office

Cisbio US
 Phone : 888-963-4567
 Fax : 781-687-1500
 E-mail : htrfinfo@cisbio.us

**Lumi4®-Tb Cryptate conjugated Streptavidin**

For in vitro research use only
 Storage temperature : 2-8°C

www.htrf.com**HTRF® package insert**

Document reference : 610SATLA/B rev00 (July 2009)

Packaging details :

	384-well low volume plate (20 µL)
610SATLA	5,000 tests
610SATLB	20,000 tests

1. Background

Streptavidin is a biotin-binding tetrameric protein isolated from *Streptomyces avidinii*. The molecular weight is about 60 kDa. There are considerable differences in the compositions of avidin and streptavidin, but they are remarkably similar in other aspects. Unlike avidin, streptavidin has no carbohydrate and has an acidic isoelectric point of 5. (10 for avidin). These two points have made streptavidin the appropriate substitute in applications for which the nonspecific binding characteristics of avidin are a problem.

Biotin, a 244 Da vitamin found in tissue and blood, binds with high affinity to streptavidin. It is the strongest known noncovalent biological interaction ($K_a=10^{15}M^{-1}$). The complexation is very rapid and, once formed, the complex is unaffected by external factors. Due to its small size, biotin can usually be conjugated to many proteins or peptides without altering significantly their biological activity.

Because of the easiness of the biotinylation and the remarkable properties of the binding, the use of streptavidin has been applied to various applications such as immunoassays, receptor-ligand interactions, protein-protein interactions, kinase activity...

This toolbox reagent has been developed for High Throughput Screening using the HTRF technology. HTRF is an homogeneous time-resolved fluorescent technique, based on the energy transfer between a long-life fluorescent cryptate donor (Europium or Lumi4-Terbium) and HTRF acceptors such as XL665, d2, or other suitable acceptor fluorophores (i.e. GFP, fluorescein...). The transferred energy is then emitted as detectable fluorescent signal. In HTRF assays, the donor and the acceptor are conjugated to biomolecules (anti-tag antibodies, streptavidins, peptides,...) for studying molecular interactions.

References:

Trinquet E. Studying molecular interactions with the new Lumi4®-Tb Cryptate HTRF toolbox. SBS 15th annual conference 2009, Lille (France).

Chaiet I and Wolf FJ. The properties of streptavidin, a biotin binding protein produced by streptomycetes. Arch Biochem Biophys. 1964;106:1-5

Green NM. Avidin. In: Advance In Protein Chemistry. New York: Academic Press. 1975;29:85-133

Mathis G. Probing molecular interactions with homogeneous techniques based on rare earth cryptates and fluorescence energy transfer. Clin Chem. 1995;41:1391-7

2. Reagent description

Streptavidin-Cryptate is produced at Cisbio Bioassays. Specific activity of the conjugate ranges from 4 to 8 Cryptates / streptavidin.

This conjugate is lyophilized in 100mM Phosphate pH 7.0, 0.1% protease free bovine serum albumin (BSA) and stabilizers.

The concentration of this conjugate has been calibrated in order to obtain a good sensitivity on HTRF assays. This calibration was run in a 384-well low volume plate format using a final volume of 20 µL, a buffer containing 0.1% BSA and 0.4M KF, and a PHERAstar Plus reader (BMG LABTECH). A recommended amount of streptavidin per well is specified in the product description sheet attached to the product. On this basis, each vial from the two available sizes enables the assessment of 5,000 and 20,000 tests respectively. Actual amount per well will be dependent on optimized assay conditions.

3. Reagent handling**3.1. Preparation of the working solution**

- Allow the lyophilized reagent to warm up at room temperature for at least 30 minutes.
- For the 5,000 test vial (610SATLA) : reconstitute the product with 250 µL of distilled water.
- For the 20,000 test vial (610SATLB) : reconstitute the product with 1 mL of distilled water.
- Vortex the solution gently.
- After reconstitution, the stock solution can be divided into aliquots and frozen at -20°C for additional use (according to storage conditions, see §5).
- Dilute with buffer the stock solution to the working concentration. Mix gently.

3.2. Recommended buffer

Most common buffers can be used for the preparation of the working solution, providing that the pH is maintained between 5.5 and 8.5. They can be complemented with BSA (0.1%) to prevent reagent coating, and detergents such as Tween 20, Triton X100, CHAPS (up to 0.5%)... may also be added. Avoid SDS, due to its denaturing effect on XL665. It is recommended to check the background signal by counting the buffer blank.

KF can play an essential role in the lanthanide cryptate protection by preventing the action of possible quenchers contained in the assay. **KF is mandatory for HTRF assays using Europium cryptate.** KF is generally used at a final concentration of 100 to 400mM, and is added to the conjugate working solutions or dispensed in a separate step, just before the readout. Assay using **Lumi4-Tb cryptate donor, does not require KF.**

4. Assay flexibility and miniaturization

When used as suggested, one vial from the two available sizes will provide sufficient reagent for 5,000 and 20,000 tests respectively using a 384-well low volume plate in 20 μ L final assay volume (HTRF[®] packaged basis).

To move to other plate formats (96 half-well or 1536-well) and final volumes (100 μ L to less than 10 μ L), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 μ L final assay volume. For instance, in the case of the 1536-well format in 10 μ L final volume, 2 times less material per well is used, thereby allowing 10,000 and 40,000 tests respectively to be run. The performances of the HTRF[®] assay remain the same whatever the level of miniaturization.

Assay components	Volume proportion	Assay format		
		1536-well (10 μ L)	384-well low volume (20 μ L)	96 half-well (100 μ L)
Other assay components	2 volume	5 μ L	10 μ L	50 μ L
Acceptor conjugate	1 volume	2.5 μ L	5 μ L	25 μ L
Cryptate conjugate	1 volume	2.5 μ L	5 μ L	25 μ L
	Small size	10,000 tests	5,000 tests	1,000 tests
	Bulk size	40,000 tests	20,000 tests	4,000 tests

96 half-well plate (Costar # 3694 or equivalent), 384-well low volume plate (Greiner # 784076), 1536-well plate (Greiner # 782086).

5. Storage conditions and stability

Lyophilized Streptavidin-Cryptate conjugate should be stored at 2-8°C until reconstituted. Under proper storing conditions, this reagent is stable until the expiry date indicated on the product description sheet.

Once reconstituted, stock solutions are stable two days at 2-8°C. They can be refrozen (at -20°C) and thawed once only. Do not repeat freezing and thawing.

Caution ! It is possible to combine donor and acceptor conjugates immediately before use. Do not store donor and acceptor conjugates mixed together for extended periods of time.