



Streptavidin-XL665

PROTOCOL

Part # 610SAXLF, 610SAXLA & 610SAXLB

Test size: 1,000 tests (610SAXLF) - 5,000 tests (610SAXLA) - 20,000 tests (610SAXLB)

Assay volume: 20 μ L

Revision: 10 (May 2018)

Store at 2-8°C

For research use only.

Not for use in therapeutic or diagnostic procedures.

REAGENT DESCRIPTION

In an HTRF protein:protein interaction assay, one protein is labeled (directly or indirectly) with the donor, and the other protein is labeled (directly or indirectly) with the acceptor. When the two proteins interact, the donor molecule is brought into proximity with the acceptor molecule. Excitation of the donor will result in signal generation proportional to the binding of proteins.

Streptavidin, the biotin-binding tetrameric protein, was labeled with XL665. Biotin, a 244 Da vitamin found in tissue and blood, binds to streptavidin with high affinity. 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.

MATERIALS

Reagent	1,000 tests	5,000 tests	20,000 tests
Streptavidin-XL665 Lyophilized In phosphate buffer pH 7.0 containing protease free bovine serum albumin.	1 vial	1 vial	1 vial

Cisbio reagents Not provided	Part #
PPI - Terbium detection buffer PPI - Europium detection buffer 200 mL - ready-to-use	Cat # 61DB10RDF* Cat # 61DB9RDF*
Plates* - HTRF 96-well low volume plate	66PL96001

*For HTRF microplate recommendations, please visit <http://www.cisbio.com/microplate-recommendations>

For reading, an HTRF®-compatible reader is needed. Make sure to use the appropriate setup.

For a list of HTRF®-compatible readers and setup recommendations, please visit <http://www.cisbio.com/readers>

STORAGE AND STABILITY

- Store the reagent at 2-8°C.
- Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.
- Once reconstituted, stock solutions are stable for two days at 2-8°C. They can be refrozen (at $\leq -60^{\circ}\text{C}$) and thawed once only.
- Do not repeat freezing and thawing.

ASSAY FORMAT

When used as suggested, one vial from the two available sizes will provide sufficient reagent for 1,000 tests, 5,000 tests and 20,000 tests respectively, using a 20 μL final assay volume. Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.

	Volume
Other assay components	10 μL
Acceptor (d2 or XL665) conjugate	5 μL
Donor (Eu or Tb Cryptate) conjugate	5 μL
Final volume*	20 μL

REAGENT HANDLING

BUFFERS

Cisbio PPI - Terbium detection buffer Cat # 61DB10RDF* and Cisbio PPI - Europium detection buffer Cat # 61DB9RDF* have been optimized for maximum performance. The buffer of choice is selected based on the donor type in the assay.

It is mandatory to use the same buffer to prepare the donor and the acceptor (d2 or XL665) conjugates.

When using specific in-house buffers for the preparation of working solutions, we recommend a basic buffer such as PBS (Phosphate Buffered Saline) or Hepes with a pH maintained between 5.5 and 8.5.

It can be supplemented with BSA (0.1%), and detergents such as Tween 20, Triton X100 or CHAPS (up to 0.5%) to prevent reagent coating. Avoid SDS, due to its denaturing effect on XL665.

Please note that a phosphate-free buffer must be used in biochemical kinase assays to prevent interferences pertaining to the binding of anti-phospho specific antibodies.

Use of Europium toolbox conjugates requires a final KF concentration between 100 mM and 400 mM. Use of Tb cryptate donor does not require KF

CONJUGATES

Allow each vial of lyophilized conjugate to warm up at room temperature.

Reconstitute the lyophilisate following the following instructions:

Streptavidin-XL665*	Stock solution preparation	Working solution preparation (see assay format above)
1,000 tests	Add 0.25 mL of distilled water Mix gently	Dilute 20-fold the stock solution in PPI detection buffer Mix gently E.g. Add 4.75 mL PPI detection buffer to 0.25 mL of stock solution
5,000 tests	Add 0.25 mL of distilled water Mix gently	Dilute 100-fold the stock solution in PPI detection buffer Mix gently E.g. Add 24.75 mL PPI detection buffer to 0.25 mL of stock solution
20,000 tests	Add 1 mL of distilled water Mix gently	Dilute 100-fold the stock solution in PPI detection buffer Mix gently E.g. Add 99 mL PPI detection buffer to 1 mL of stock solution

*Additional info is included in the batch information provided with the reagent.

The optimal amount per well will be dependent on assay conditions. For additional information on assay optimization, please refer to the technical note: [Guidelines for optimizing protein : protein interaction assays using HTRF PPI reagents.](#)

Make sure to prepare stock and working solutions according to the instructions that correspond to the packaging you have purchased (number of tests).

This product contains material of biological origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.

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