

Instruction for Use

Exazym® StreptaClick® Primer

Catalog Number 10-0002-01

For research use only. Not for use in diagnostic procedures.

This Instruction for Use (IFU) describes how to store, prepare, and correctly use Exazym® StreptaClick® Primer (cat no. 10-0002-01). The product contains the reagent required to implement the BOLD technology in an immunoassay using a biotin-conjugated detector antibody. This product is used together with the Exazym® Polymerase Reaction Kit and Exazym® detection kits and enables signal amplification of immunoassays.

Introduction to BOLD

Measurement of low abundance biomolecules remains a challenge in many pre-clinical, clinical, and diagnostic applications due to insufficient sensitivity. While some biomarker discovery and detection as well as clinical diagnostic measurement methods have made significant advances in sensitivity, there are still many potential disease biomarkers that exist in accessible biofluids at levels below the detection limits or where an increased precision is desirable. Furthermore, many methods require specialized instruments, increasing the cost and logistical complexity of large-scale adoption.

Cavidi has developed a range of Exazym® reagents and kits based on its proprietary BOLD signal amplification technology. BOLD brings ultra-sensitive detection levels to conventional immunodiagnostic assays, and "BOLD" stands for Binding Oligo Ladder Detection. In BOLD, a detector antibody conjugated to an oligo-dT primer is first bound to its target. A polymerase with reverse transcriptase activity, BrdUTP monomers (5-Bromo-2'-deoxyuridine 5'-triphosphate), and a rA-template are then added, extending the primer into a long DNA:RNA hybrid-ladder. Anti-BrdU detection antibodies bind this ladder selectively to generate the amplified signal. An alternative method is available for users working with biotinylated detector antibodies. A proprietary streptavidin-primer molecule binds directly to the biotin, removing the conjugation step. However, direct conjugation may be preferable when fewer incubation steps are needed, when biotin interference is a concern (e.g., samples with high endogenous biotin), or when the detector-antibody:primer ratio must be optimized. Integration of BOLD into an immunoassay enhances sensitivity, typically improving the limit of detection ranging from 10-100x when used with either Exazym® StreptaClick® Primer or Exazym® ClickChem Conjugation kit.

Signal enhancement using BOLD and the Exazym® kit system is useful for signal enhancement of immunoassays, particularly in translational research, health screening and diagnostic testing. Examples of such applications are biomarker discovery and detection, especially in the field of low abundance proteins, *in vitro* diagnostics in the fields where improved detection levels are crucial such as neurology, cardiology, and monitoring of relapse in previously treated cancer patients.

What is Exazym® StreptaClick® Primer?

Exazym® StreptaClick® Primer is a component of the Exazym® system that simplifies the implementation of BOLD for users working with biotinylated detector antibodies. It is based on a proprietary streptavidin molecule (StreptaClick®, Kromnigon) conjugated to the oligo-dT primer, which binds directly to the biotin on the detector antibody.

As such, the Exazym® StreptaClick® Primer is the first step in an Exazym workflow comprising:

- **Exazym® StreptaClick® Primer** – for direct binding of the oligo-dT primer to the biotinylated detector antibody.
- **Exazym® Polymerase Reaction Kit 96 and 960** – for performing the polymerase reaction when applying BOLD technology for signal amplification of immunoassays.

- **Exazym® Detection Kits 96 and 960** – for detection of the DNA:RNA hybrid using an anti-BrdU antibody conjugated to the detection moiety of your choice, e.g., Biotin, HRP, PE, APC (see www.cavidi.se for available options).

Exazym® StreptaClick® Primer allows assay developers to deploy the full sensitivity advantage of BOLD with no modification to existing antibody reagents. It is intended for research use only and must not be used in diagnostic procedures.

Product specification

The product contains the streptavidin-oligo-dT primer required for polymerization of the DNA:RNA hybrid-ladder using the Exazym® Polymerase Reaction Kit. The reagent supplied is sufficient for at least one 96-well microplate. The Exazym® system can bring ultra-sensitive detection levels to conventional immunodiagnostic assays.

Components included

Exazym® StreptaClick® Primer contains the following components:

- 1 vial x 2.5 µg Exazym® StreptaClick® Primer, 0.1 mg/mL in TE buffer.

Materials and equipment required but not included

- Exazym® Polymerase Reaction Kit 96 or 960
- Exazym® Detection Kit 96 or 960 (select the detection moiety appropriate for your assay; see options above)
- Exazym® Wash Buffer, which is provided as blister tablets with Exazym® Detection Kit 96 and 960.
- ELISA diluent for dilution of Exazym® StreptaClick® Primer (e.g., PBS-based buffer at neutral pH containing stabilizer and detergent)
- Adhesive plate covers
- Plate washer; automated or manual (e.g., multi-pipette)
- Pipettes and other standard laboratory equipment

Shipping conditions

Shipped on wet ice.

Storage conditions

Exazym® StreptaClick® Primer is stored at +2 °C to +8 °C.

Warnings



Do not use a product that is damaged upon arrival. Please contact customer support immediately (see below for contact details).

This product is for research and laboratory use only and must be handled by professional and trained users only.

Personal protective equipment, such as eye protection and gloves, must be worn when handling the kit.

For further safety information, please refer to the Safety Data Sheet on Cavid's web page or call customer support (see below for contact details).

How to use Exazym® StreptaClick® Primer

First-time use – optimization of Exazym® StreptaClick® Primer and biotin-detector antibody concentration

1. For first-time use, it is recommended to perform a cross-titration to find the optimal concentrations of the Exazym® StreptaClick® Primer reagent and your biotin-detector antibody. Recommended concentration range of your biotin-detector antibody is 0.125x - 0.5x of your standard immunoassay protocol. The recommended range for Exazym® StreptaClick® Primer is 10–100 ng/mL, but it must be optimized for your specific assay. Figure 1 shows a suggested microtiter plate layout as a starting point. The layout uses only part of a 96-well plate, so it is compatible with strip-based plates and leaves unused strips available for samples or for additional antibody and primer combinations. Further optimization can then be performed around the best-performing conditions.
2. Exazym® StreptaClick® Primer can be diluted in your standard ELISA diluent (should not contain biotin as this will affect the function of the reagent), e.g. PBS at neutral pH containing a stabilizer and detergent.

	1	2	3	4	5	6	7
A	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1	Std 1
B	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2	Std 2
C	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3	Std 3
D	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4	Std 4
E	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5	Std 5
F	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6	Std 6
G	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7	Std 7
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Assay Type:	Reference ELISA	BOLD amplified ELISA					
Biotin-detector Ab:	1x		0,5x		0,25x		
Exazym StreptaClick Primer:	100 ng/ml	10 ng/ml	100 ng/ml	10 ng/ml	100 ng/ml	10 ng/ml	

Figure 1. Suggested plate layout for first-time concentration optimization with Exazym® StreptaClick® Primer. The 1x, 0.5x, and 0.25x values refer to the concentration of the biotin-detector antibody relative to its standard immunoassay protocol. The layout uses only part of a 96-well plate and is compatible with strip-based plates; unused strips can be used for samples or for additional antibody and primer combinations (e.g., 0.125x antibody with 100 and 10 ng/mL Exazym® StreptaClick® Primer).

Preparation before use – Exazym® Wash Buffer

1. Prepare Exazym® Wash Buffer by dissolving one tablet in 1 L deionized water using a laboratory flask or beaker and a magnetic stirrer. Stir until fully dissolved.
2. Allow Exazym® Wash Buffer to reach room temperature.

Procedure for binding Exazym® StreptaClick® Primer to the biotin-detector antibody

Perform your standard assay protocol using your assay-specific concentration of the biotinylated detector antibody (see the First-time use section above). After incubation of your biotin-detector antibody, wash the wells and proceed with the Exazym® StreptaClick® Primer protocol below.

1. Briefly centrifuge the microcentrifuge tube before use to collect the liquid at the bottom.
2. Prepare the Exazym® StreptaClick® Primer working solution by diluting it to your assay-specific concentration (see the First-time use section above) using your standard ELISA diluent.
3. Add 100 µL/well of the working solution.

4. If a plate-based system is used, cover the plate with an adhesive plate cover.
5. Incubate for 10 min at room temperature.
6. Wash 3x300 µL/well with room-temperature Exazym® Wash Buffer.
7. Proceed to the next step, polymerization of the DNA:RNA hybrid, using the Exazym® Polymerase Kit 96 or 960.

Note: If you have any comments or questions or would like to discuss protocol optimization with one of Cavidí's technical experts, please do not hesitate to contact us (see below for contact details).

Intellectual Property Rights

The use of this product may be patent protected (PCT WO 2022/250596 A1). Any use except for research purposes requires a license from Cavidí AB, Virdings Allé 2 SE-754 50 Uppsala, Sweden. For further information and license terms and conditions, please contact Cavidí AB at info@cavidi.se or +46 (0)707 38 07 29.

How do I get in contact with Cavidí's tech support?

In case you have any inquiries or technical support questions, please contact us at support@cavidi.se or +46 (0)707 38 07 29.

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