Instruction for Use

Exazym® Biotin Detection Kit 960

Catalogue Number 10-2001-02

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

This Instruction for Use (IFU) describes how to store, prepare, and correctly use the Exazym® Biotin Detection Kit 960 (cat no. 10-2001-02). The kit contains the reagents required to implement the detection step of the BOLD technology in an immunoassay. This step is preceded by the use of Exazym® ClickChem Conjugation Kit and Exazym® Polymerase Reaction Kit. To detect the signal amplification generated by BOLD, a final detection modality, e.g. streptavidin-enzyme or streptavidin-fluorescent dye or other conjugates of your choice, will be attached to Exazym® Antibody Biotin. For example, streptavidin-horseradish peroxidase may be used if a colorimetric detection modality is preferred.

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 of these techniques or where an increased precision is desirable. Furthermore, they 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. When using BOLD a oligo-dT primer conjugated to a secondary detection antibody is mixed with a polymerase containing reverse transcriptase activity, 5-Bromo-2'-deoxyuridine 5'-triphosphate monomers and a rA-template to create the BOLD construct, a long hybrid-ladder of DNA:RNA, to which biotinylated anti-BrdU detection antibodies selectively bind. Integration of BOLD into an immunoassay improves the sensitivity and the limit of detection can be improved by up to 50x.

Signal enhancement using BOLD and Exazym® Kit System is useful for signal enhancement of immunoassays in particularly in translational research, health screening and diagnostics 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 already treated cancer patients.

What is Exazym® Biotin Detection Kit 960?

Exazym® Kit System consists of three products necessary to perform BOLD; i) conjugation of a primer to the detector antibody, ii) the polymerization phase to generate the hybrid cDNA and, iii) binding of secondary antibodies to the BOLD construct to provide the final signal. All required components for these three unique events of BOLD are included in:

- Exazym® ClickChem Conjugation Kit 50 and 250 - for conjugation of Exazym® primer to the detection antibody of choice.
- Exazym® Polymerase Reaction Kit 96 and 960 - for performing the polymerase reaction when applying BOLD technology for signal amplification of immunoassays.
- Exazym® Biotin Detection Kit 96 and 960 - for detection of hybrid strands by a report antibody; Exazym® Antibody Biotin.

Exazym® Biotin Detection Kit 960 is intended for detection of the long hybrid-ladder consisting of the poly-rA and the complementary poly-BrdU strands. The kit is for research use only and shall not be used in diagnostic procedures.

Product specification

The kit contains the antibody and buffers required for detection of the poly-rA:poly-BrdU hybrid strand polymerized using Exazym® Polymerase Reaction Kit.

The kit contains the reagents required to implement the detection step of the BOLD technology in an immunoassay. The 960 kit contains reagents for 10 microplates x 96 wells. This step is preceded by the use of Exazym® ClickChem Conjugation Kit and Exazym® Polymerase Reaction Kit. To detect the signal amplification generated by BOLD, a final detection modality, e.g. streptavidin-enzyme or streptavidin-fluorescent dye or other conjugates of your choice, will be attached to Exazym® Antibody Biotin. For example, streptavidin-horseradish peroxidase may be used if a colorimetric detection modality is preferred.

Components included in the kit

The kit contains the following components:

2 vials 25 µg Exazym® Antibody Biotin (biotinylated anti-BrdU monoclonal antibody), 0.5 mg/ml

1 vial 115 ml Exazym® Antibody Buffer

5 tablets Exazym® Wash Buffer to be reconstituted in 1 litre de-ionized water per tablet

Materials and equipment required but not included

Streptavidin-HRP with appropriate substrate system or the reagents required for an alternative detection modality

De-ionized water

Plate washer; automated or manual (e.g. multi-pipette)

Plate reader for detection system of choice

Pipettes and other standard laboratory equipment

Shipping conditions

Shipped on wet ice.

Storage conditions

Exazym® Antibody Biotin is stored at +4-8°C.

Exazym® Antibody Buffer is stored at -20°C.

The tablets of Exazym® Wash Buffer are stored in a dry place at room temperature. After reconstitution in de-ionized water, store the liquid at +4-8°.

Shelf life

18 months from date of manufacture. Please refer to the secondary kit packing label for the manufacturing date.

Warnings

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

Personal protective equipment shall be used such as eye shield and gloves when handling the kit.

This is a single-use kit. Please follow the instructions for use and discard any used and old vials or chemicals. Do not re-use any vials or chemicals.

Do not use a kit which is broken upon arrival. Please contact customer support immediately (see below for contact details).

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

How to use Exazym® Biotin Detection Kit 960?

Preparations before use – Exazym® Antibody Biotin Working Solution

1. Before use, allow Exazym® Antibody Buffer to reach room temperature.

2. Prepare and dilute Exazym® Antibody Biotin to a final concentration of 0.3 µg/ml using the antibody stock solution supplied with the kit and the Exazym® Antibody Buffer which has been allowed to reach room temperature (see step 1 above). Pre-incubate the antibody Working Solution for 1 hour at room temperature.

Preparation before use - Exazym® Wash Buffer

1. Prepare Exazym® Wash Buffer by dissolving each tablet in 1 litre de-ionized water using a laboratory flask or beaker and a magnetic stirrer. The solution shall be stirred until no debris is left and the solution is clear. The kit contains 5 tablets.

2. Pre-incubate Exazym® Wash Buffer to reach room temperature.

Procedure for detection of the poly-rA:poly-BrdU hybrid strand

1. Add 100 µl/well of Exazym® Antibody Biotin Working Solution at a concentration of 0.3 µg/ml prepared as described in step 2 above.

2. If a plate-based system is used, cover the plate with an adhesive plate cover.

3. Incubate for 30 minutes at room temperature.

4. Wash 3 times with Exazym Wash Buffer which has been allowed to reach room temperature.

5. Proceed with next step which is detection of Exazym® Antibody Biotin by Streptavidin-HRP conjugate and thereafter in-house detection method.

6. At this step, use your own reagents and conjugates to detect the tertiary antibody and follow their specific IFU.

NOTE: The amount of Exazym® Antibody Biotin supplied is greater than required for the procedure above. Hence, higher and/or lower concentration than the recommended concentration of 0.3 µg/ml may be tested. Optimal concentration might vary depending on which system BOLD has been applied. In case a short assay time is critical, a shorter Exazym® Antibody Biotin incubation time may be tested. An incubation time as short as 5 minutes may be sufficient depending on the ELISA system.

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 Cavidi AB, Virdings Allé 2 SE-754 50 Uppsala, Sweden. For further information and license terms and conditions, please contact Cavidi AB at info@cavidi.se
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How do I get in contact with Cavidi's tech support?

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

Cavidi AB (Head Office)
Virdings Alle' 2
SE-754 50 Uppsala
Sweden
TEL: +46 (0)18 55 20 40
info@cavidi.se

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