Instruction for Use

Exazym® Polymerase Reaction Kit 960

Catalogue Number 10-1001-02

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

This Instruction for Use (IFU) describes how to store, prepare and correctly use Exazym® Polymerase Reaction Kit 960. The kit contains reagents to perform 960 polymerase reactions (10 x 96) in parallel. The kit is used together with a secondary antibody to which an oligo-dT primer has been conjugated using Exazym® ClickChem Conjugation Kit 50 or 250 and Exazym® Biotin Detection Kit 96 or 960. The Exazym® system has been designed for signal amplification of immunoassays using Cavidi's proprietary BOLD technology.

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 an 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 (BrdUTP) and a rA-template to create a long hybrid-ladder of DNA:RNA to which biotinylated anti-BrdU detection antibodies selectively bind to enable the amplified signal. 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® Polymerase Reaction Kit 960?

Exazym® Kit System consists of three products necessary to perform BOLD; i) conjugation of a primer to the detector antibody (secondary antibody), ii) the polymerization phase to generate the hybrid ladder of DNA:RNA and, iii) binding of the biotinylated anti-BrdU detection antibodies to the hybrid ladder of DNA:RNA 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 the DNA:RNA hybrid by a biotinylated anti-BrdU antibody; Exazym® Antibody Biotin.

Exazym® Polymerase Reaction Kit 960 is intended for signal enhancement of immunoassays. A hybrid-ladder of DNA:RNA is polymerized using an oligo-dT primer conjugated to a secondary antibody. The oligo-dT primer is extended using a polymerase having reverse transcriptase activity, BrdUTP monomers as substrate and a poly-rA as the template. The kit is designed to support assay optimisation work or large scale automated routine workflows. The kit is intended for research use only and not for diagnostic procedures.

Product specification

The reagents required for polymerisation of a long hybrid-ladder of DNA:RNA on an oligo-dT primer conjugated secondary antibody. Exazym® Polymerisation Reaction Kit 960 is designed to be used together with Exazym® ClickChem Conjugation Kit 50 or 250 and Exazym® Biotin Detection Kit 96 or 960 and can bring ultra-sensitive detection levels to conventional immunodiagnostic assays.

Components included in the kit

The kit contains the following components:

1 vial 100 units of Exazym® RT Polymerase

1 vial 40 mL Exazym® Reaction Solution

1 vial 100 mL Exazym® Polymerase Buffer

1 vial 900 µL Exazym® Template (1 mg/mL)

Materials and equipment required but not included

Adhesive plate covers

De-ionized water

Exazym® Wash Buffer which is provided with Exazym® Biotin Detection Kit 96 and 960 as blister tablets.

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

Pipettes and other standard laboratory equipment

Shipping conditions

Shipped on wet ice.

Storage conditions

The kit and all components of the kit shall be stored at -20 °C.

Exazym® Polymerase Buffer, Exazym® Reaction Solution and Exazym® Template may be aliquoted and stored at -20 °C.

Warnings

RUO

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

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.

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® Polymerase Reaction Kit 960

Preparation before use – Exazym® Wash Buffer

1. Prepare Exazym® Wash Buffer by dissolving each tablet in 1 L of de-ionized water using a laboratory flask or beaker, a magnetic rod and a magnetic stirrer.

Note: Exazym® Wash Buffer is provided with Exazym® Biotin Detection Kit 96 and 960 as blister tablets.

Preparation before use – Exazym® Reaction Solution and Exazym® Template mix

1. Shortly before use, allow Exazym® Reaction Solution and Exazym® Template to reach room temperature.

2. For every mL of Exazym® Reaction Solution add 22 µL of Exazym® Template. For a 96 wells system 23 µL/well is needed.

Preparation before use – Exazym® Polymerase Working Solution

1. Allow Exazym® Polymerase Buffer to reach room temperature.

2. Shortly before use, prepare Exazym® RT Polymerase Working Solution by diluting it with Exazym Polymerase Buffer to 0.65 U/mL. The concentration of the supplied Exazym® RT Polymerase stock solution may vary between lots and pack sizes. The concentration of the working solution shall be 0.65 U/mL. For a 96 wells system 77 µL/well is needed.

Procedure for polymerization of the hybrid-ladder of DNA:RNA

The procedure below assumes that the initial steps of the immunoassay have been performed. The primary antibody has caught the antigen, the secondary antibody, conjugated with the oligo-dT primer, has bound to the antigen and the unbound antibodies have been washed away.

1. In a 96 wells plate system add 23 µL/well of Exazym® Reaction Solution (mixed with Exazym® Template) followed by 77 µL/well of Exazym® RT Polymerase Working Solution.

2. Place an adhesive plate cover over the plate and incubate at room temperature for 10-30 minutes. The optimal polymerization time may vary depending on the antibody/antigen system to which BOLD has been applied. To optimize the specific assay, you may test longer polymerization times (up to one hour).

3. Wash 3x300 µL/well with Exazym® Wash Buffer (provided in Exazym® Biotin Detection Kit 96 and 960).

4. To detect the polymerized BOLD construct, proceed with Exazym® Biotin Detection Kit. For more information about these kits read the instruction for Exazym® Biotin Detection Kit 96 or 960 (Catalog Numbers 10-2001-01 and 10-2001-02, respectively).

Note: If you have any comments or questions or would like to discuss protocol optimisation with one of Cavidi'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 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 or +46 (0)707 38 07 29.

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.

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