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# **Instruction for Use**

## **Exazym® HRP Detection Kit 96**

### **Catalogue Number 10-2005-01**

**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® HRP Detection Kit 96 (cat no. 10-2005-01). 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.

### **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 detector 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 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® HRP Detection Kit 96?**

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 ladder of DNA:RNA and, iii) binding of the HRP 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 detector 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® HRP Detection Kit 96 and 960 - for detection of the DNA:RNA hybrid by a Horse Radish Peroxidase (HRP) conjugated anti-BrdU antibody; Exazym® Antibody HRP.

Exazym® HRP Detection Kit 96 is intended for detection of the long DNA:RNA 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 DNA:RNA hybrid-ladder polymerized using Exazym® Polymerase Reaction Kit. The reagents supplied with the kit are sufficient for 1 microplate x 96 wells. Please note that the kit does not contain a substrate. The kit can bring ultra-sensitive detection levels to conventional immunodiagnostic assays.

## Components included in the kit

The kit contains the following components:

1 vial 5 µg Exazym® Antibody HRP (HRP conjugated anti-BrdU monoclonal antibody), 0.5 mg/mL. Contains 0.05% Proclin™ 300 as preservative.

1 vial 12 mL Exazym® Antibody Buffer. Contains 0.05% Proclin™ 300 as preservative.

1 tablet Exazym® Wash Buffer to be reconstituted in 1 L de-ionized water

## Materials and equipment required but not included

Adhesive plate covers

Substrate for HRP, and stop solution if applicable

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 HRP is stored at +2 °C to +8 °C.

Exazym® Antibody Buffer is stored at +2 °C to +8 °C.

The tablet of Exazym® Wash Buffer is stored in a dry place at room temperature or at +2°C to +8°C. After reconstitution in de-ionized water, store the liquid at +2 °C to +8 °C.

## Warnings



This is a single-use kit. Please follow the instructions below 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).

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 Cavidí's web page or call customer support (see below for contact details).

## How to use Exazym® HRP Detection Kit 96

### Preparations before use – Exazym® Antibody HRP Working Solution

1. Before use, allow Exazym® Antibody Buffer to reach room temperature.
2. Prepare and dilute Exazym® Antibody HRP 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 HRP Working Solution for 1 hour at room temperature.

### Preparation before use – Exazym® Wash Buffer

1. Prepare Exazym® Wash Buffer by dissolving one tablet in 1 L de-ionized water using a laboratory flask or beaker and a magnetic stirrer. The solution shall be stirred until fully dissolved.
2. Allow Exazym® Wash Buffer to reach room temperature.

### Procedure for detection of the DNA:RNA hybrid-ladder

1. Add 100 µL/well of Exazym® Antibody HRP Working Solution at a concentration of 0.3 µg/mL prepared as described above.
2. If a plate-based system is used, cover the plate with an adhesive plate cover.
3. Incubate for 30 min at room temperature.
4. Wash 3x300 µL/well with Exazym Wash Buffer which has been allowed to reach room temperature.
5. Proceed with next step which is detection using the HRP substrate of choice.
6. At this step, use your own reagents and follow their specific IFU.

**Note:** In case a short assay time is critical, a shorter Exazym® Antibody HRP incubation time may be tested. An incubation time as short as 5 min may be sufficient depending on the ELISA system. If you have any comments or questions or would like to discuss protocol optimisation with one of Cavidis' 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 Cavidis AB, Virdings Allé 2 SE-754 50 Uppsala, Sweden. For further information and license terms and conditions, please contact Cavidis AB at [info@cavidis.se](mailto:info@cavidis.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](mailto:support@cavidi.se) or +46 (0)707 38 07 29.

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