Instruction for Use

Exazym® APC Detection Kit

Catalogue Number 10-2002-01

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® APC Detection Kit (cat no. 10-2002-01). 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.

Different types of Exazym detection kits, containing anti-BrdU antibodies conjugated with different labels, are available.

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® APC Detection Kit?

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 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® APC Detection Kit - for fluorescence detection of the DNA:RNA hybrid by an allophycocyanin (APC) conjugated anti-BrdU antibody; Exazym® Antibody APC.

Exazym® APC Detection Kit is intended for APC fluorescence detection of the long DNA:RNA hybrid-ladder consisting of the poly-rA and the complementary poly-BrdU strands. APC has an excitation max at 651 nm and an emission max at 660 nm, similar to the excitation and emission properties Alexa Fluor™ 647. This APC detection kit is suitable for fluorescence detection in general immunoassays requiring these fluorescent properties as well as compatible with specialized systems

like the Gyrolab system or SMCxPRO. 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 APC fluorescence detection of the DNA:RNA hybrid-ladder polymerized using Exazym® Polymerase Reaction Kit. 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 8 µg Exazym® Antibody APC (APC conjugated anti-BrdU monoclonal antibody), in Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)

1 vial 12 mL Exazym® Antibody Buffer

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

Note: The consumption of the different components can vary depending on the application. In case you need more wash buffer PBS 0.05% Tween20 pH 7.4 can be used. In case you need more Exazym Antibody Buffer contact Cavidi and request to buy the buffer separately.

Shipping conditions

Shipped on wet ice.

Storage conditions

Exazym® Antibody APC 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

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® APC Detection Kit

Preparations before use – Exazym® Antibody APC Working Solution

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

2. Prepare and dilute Exazym® Antibody APC to working concentration 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). The antibody stock solution concentration may vary between lots and is specified on the vial. Recommended working concentration is around 0.3-0.6 µg/mL. The optimal working concentration should however be determined empirically for each specific application and could be outside the recommended range.

Note: A pre-incubation of the antibody working solution for 1 hour at room temperature and protected from light may decrease background noise.

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

The procedure below assumes that the initial steps of the immunoassay have been performed and that the DNA:RNA hybrid-ladder has been formed by following the instructions for Exazym Polymerase Reaction Kit.

1. Add the Exazym® Antibody APC Working Solution prepared as described above. The volume of Working Solution to use should be adjusted according to volumes used in previous steps in the immunoassay and depend on the application.

2. Incubate for 30 min at room temperature.

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

4. Proceed with fluorescence detection.

Note: In case a short assay time is critical, a shorter Exazym® Antibody APC incubation time may be tested. 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

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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