



Immune Complex Dissociation Kit

Catalog# XB-2000

Introduction

Principle of the Assay

Some ELISA assays are used to detect antigens in various matrices such as tissue culture media and serum or plasma. If the assay is used in serum or plasma, it may yield a false negative result due to circulating antibodies producing immune complexes. This kit contains two reagents that supplement the XpressBio HCV Core ELISA kit (XC-1000) and HIV-1 p24 ELISA Kit (XB-1000). They are used to dissociate the immune complexes and allow the HCV core to be detected.

Kit Presentation

Materials Supplied

The reagents supplied in this pack are for **Research Use Only**.

1	Immune Complex Reagent A	10 ml
2	Immune Complex Reagent B	10 ml

Additional Requirements for Manual Processing

- > Disposable tip micropipettes to deliver volumes of 5 μ L, 10 μ L, 25 μ L, 100 μ L and 200 μ L (multichannel pipette preferred for dispensing reagents into microtiter plates).
- > Distilled or deionized water.
- > 37 (\pm 1) ° C incubator.
- > Clean, disposable plastic/ glass test tubes, approximate capacities 5 mL and 10 mL.
- > Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL glass pipettes.
- > Absorbent paper towels.
- > Automatic microtitration plate washer or laboratory wash bottle.
- > Microtitration plate reader with 450 nm filter.
- > Latex gloves, safety glasses and other appropriate protective garments.
- > Biohazard infectious waste containers.
- > Safety pipetting devices for 1 mL or larger pipettes.
- > Timer.

Storage and Stability

All reagents should be stored at 2-8°C, and should not be used beyond the expiration date on the label.

Warnings and Precaution

Safety

1. The reagents supplied in this kit are for **Research Use Only**.
2. Caution: All blood products should be treated as potentially infectious.

Essential precautions can be summarized as follows:

- >Do not pipette by mouth.
 - >Wear disposable gloves during all specimen and assay manipulations.
 - >Avoid use of sharp or pointed liquid handling devices, which may puncture skin.
 - >Do not smoke, eat or drink in the laboratory work area.
 - >Avoid splashing of liquid specimens and reagents and the formation of aerosols.
 - >Wash hands thoroughly on completion of a manipulation.
 - >The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Bio Safety Level 2.
3. The ELISA kits contain reagent systems, which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.

Procedural

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use kits after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
4. Always use clean, preferably disposable, glassware for all reagent preparation.
5. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
6. Always keep the upper surface of the microtitration strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
7. Do not allow the wells to completely dry during an assay.
8. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
9. Automatic or semi-automatic EIA processors or liquid handling systems should be qualified specifically for use with HCV Core Assay by demonstration of equivalence to the manual processing methods.
10. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
12. Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Method of Use

Specimen Storage

This Assay can be used for serum and plasma with these reagents. The specimen should be tested as soon as possible. However, if the specimen needs storage, the specimens should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Specimens that have been frozen and thawed should be thoroughly mixed before testing.

Preparation for the Assay

1. *XpressBio HCV Core ELISA kit (XC-1000) Kit Positive Control 100 ng/ml*
or XpressBio HIV-1 P24 ELISA kit (XB-1000) Kit Positive Control 100 ng/ml

Prepare working strength Positive Control by diluting 20 μ L of positive control into 980 μ L (1:50 dilution) of uninoculated tissue culture media or negative normal serum or plasma. This will give a final concentration of 2000 pg/ml.

Qualitative Assay Procedure

1. Allow all reagents to reach room temperature (18-25°C).
2. The diluted positive control (2000 pg/ml) and uninoculated cell culture media or normal serum or plasma (for use as a negative control) should be tested at least in duplicate in every assay. If a standard curve is to be run, the quantitative protocol should be used.
3. Dispense 75 μ L of Positive Control and 75 μ L of Immune Complex reagent A into appropriate tubes and mix. Prepare the negative control and all samples in the same way.
4. Incubate at 37(\pm 1) °C for 60 (\pm 5) minutes.
5. Dispense 75 μ L of Immune Complex reagent B into each tube and mix.
6. Assay each specimen using the XpressBio HCV Core ELISA kit (XC-1000) using the procedure outlined in the kit.

Quantitative Assay Procedure

To test quantitatively, a standard curve should be prepared using tissue culture media or normal serum or plasma as the diluent. First prepare the 2000 pg/ml standard as above in step 1. Prepare four serial two fold dilutions to prepare 1000 pg/ml, 500 pg/ml, 250 pg/ml and 125 pg/ml standards using the tissue culture media as diluent. Each standard plus an inoculated tissue culture control should be run in duplicate.

Interpretation of Results

Qualitative Analysis

The following criteria should be met for a valid assay:

The negative control should be ≤ 0.10

The 1000 pg/ml control should be ≥ 0.40



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