



## **Clostridium difficile GDH CLIA Kit**

### **Instructions for Use (IFU)**

**For Research Use Only. Not for use in diagnostic or therapeutic procedures.**



**80-GDHHU-FC100**

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## Intended Use

The Clostridium difficile GDH CLIA Kit is an automated immunoassay intended for use by trained laboratory personnel for the qualitative determination of *Clostridium difficile* glutamate dehydrogenase (GDH) in human stool using the KleeYa platform. For Research Use Only. Not for use in diagnostic procedures.

## Principles of the Procedure

The Clostridium difficile GDH CLIA Kit is a chemiluminescent immunoassay system with detection based on the use of a monoclonal antibody against the *Clostridium difficile* GDH. The KleeYa system allows for complete automation of immunoassays. It performs the sample processing (sample pre-dilutions, sample and reagent dispensing, incubations, wash processes) as well as the measurement and evaluation. A monoclonal capture antibody (mAb), highly specific to *C. difficile* GDH, is coated onto the magnetic beads. *C. difficile* present in the sample extract is bound by the antibody conjugated to the surface of the magnetic beads. Re-calibrator, controls and sample extracts are incubated with a chemical-conjugated monoclonal antibody that detects the *C. difficile* GDH bound to the antibody-coated magnetic beads. After incubation and a wash step, a substrate is added. Upon reaction, a flash of signal is directly read on the KleeYa instrument. The intensity of the light is proportional to the amount of conjugate bound, and thus to the amount of captured *C. difficile* GDH. The presence or absence of *C. difficile* GDH is determined by comparison to a cut-off re-calibrator.

## Reagents / Materials Provided

The *C. difficile* GDH CLIA Kit is specifically designed for automated analysis using the KleeYa instrument. To perform analysis on the instrument, carefully follow maintenance and operating instructions in the KleeYa user manual.

## Reagents Supplied for 100 Tests

<b>REAGENT CARTRIDGE</b>	<b>Magnetic Beads</b> 1 vial of magnetic beads conjugated with monoclonal antibody against <i>C. difficile</i> GDH. Ready to use.	1 x 5mL
	<b>Conjugate</b> Diluted enzyme-conjugated monoclonal detector antibody against <i>C. difficile</i> GDH. Ready to use.	2 x 10mL

## Ancillary Materials

### \*USB Storage Device (10-CLUSB-01)

\*Each kit lot is supplied with a Universal Serial Bus (USB) Storage Device containing the lot-specific Definition file (a parameter file defining all the automated steps of the assay). The data from the USB Storage Device is uploaded onto the KleeYa via the USB port prior to assay run. Please refer to the instructions enclosed with the USB Storage Device (10-CLUSB-01) for uploading of lot-specific assay files.

## Reagents and Consumables Required but Not Provided

<b>RECALIBRATOR</b>	<b>Cut-Off Re-calibrator</b> 1 vial containing <i>C. difficile</i> GDH at a fixed concentration. Ready to use.	1 x 1mL
<b>CONTROLS</b>	<b>Pos and Neg Controls</b> 2 vials, each containing <i>C. difficile</i> GDH at a fixed concentration. Ready to use.	2 x 1mL
<b>15-10111184</b>	KleeYa Trigger Pack 3 sets of trigger solutions	3,000 tests
<b>15-10111002</b>	Wash Buffer 5X	4 x 2L
<b>15-10033666</b>	Stackable Cuvettes; 1,000 µL	3,000 cuvettes
<b>15-10036506</b>	Disposable Anchor Tips; 300 µL	5,760 tips

## Additional Materials Required for Sample Collection and Extraction but Not Provided

### Feces sample collection

1. Sample collection tube
2. Transport container

### Manual feces preparation

1. Liquid Samples: Suitable pipette for liquid stool transfer
2. Solid Samples: Disposable, breakable sterile inoculation loops or wooden stick
3. Solid Samples: Sensitive digital scale (40-150 mg)
4. Disposable polypropylene screw cap tubes, 14 ml
5. Eppendorf tubes (1 – 1.5 ml)
6. Extraction buffer 4X concentrate (10-EXBUF-225)
7. Vortex mixer or shaker
8. Centrifuge (3,000 x g)
9. Freezer (-80°C)

### Device Extraction Method:

1. Easy Stool Extraction Device (80-EZEX2-100) or comparable validated device
2. Eppendorf tubes (1 - 1.5 ml) or microcentrifuge tubes (2 mL)
3. Vortex mixer or shaker
4. Centrifuge (3,000 x g)
5. Freezer (-80°C)

## Precautions and Warnings

1. For research use only.
2. Follow universal precautions. Materials of human origin used in this kit have been tested and confirmed negative for HBsAg and anti-HIV I and II and anti-HCV antibodies. They should be treated as a potential biohazard and handled and disposed of according to local laboratory legislation.
3. Re-calibrators and controls may only be run with corresponding reagents. If purchased separately, the lot number for the re-calibrator and control set (80-GDHHU-FCSET) must match the lot number for the reagent kit (80-GDHHU-FC100) being run.

4. Routine maintenance of the KleeYa instrument is required for proper performance.
5. All reagents contain ProClin 300 as a preservative agent (< 0.10%).
6. The ALPCO extraction device should not be used for the extraction of liquid/watery stool samples. Samples of this consistency should be extracted using the manual weighing extraction method.
7. Stool samples are generally heterogeneous. Mechanical homogenization using an applicator, inoculation loop, or similar device prior to sampling is recommended.
8. EASY 2 Stool Extraction Devices may only be loaded into KleeYa racks containing the small black spacers. Re-calibrators and controls may only be loaded into a KleeYa sample rack containing white spacers.
9. Additional safety information on hazardous substances included in the kit and actions to take in the event of direct contact or ingestion is provided within the Safety Data Sheet (SDS) for this product. To obtain an SDS, please contact our customer service department at (800) 592-5726 or email: [ts@alpc.com](mailto:ts@alpc.com).
10. Dispose of unused reagents according to local and federal regulations.

### Storage and Transport Conditions

1. Reagents are shipped on cold packs but do not require specific temperature control. Reagents should be stored at 2-8°C. Unopened reagents are stable until the expiration date on the box label. **Do Not Freeze.**
2. The expiration date is printed on all component labels.
3. When cartridges are not in use, it is recommended they are stored at 2-8°C.

### Reagent Stability

<b>Reagent Cartridges</b>
Store at 2-8°C. Unopened cartridges are stable until the expiration date printed on the label. Opened cartridges are stable for up to 28 days.
<b>Re-calibrators and Controls</b>
Store at 2-8°C. Unopened re-calibrators and controls are stable until the expiration date printed on the label. Opened re-calibrators and controls are stable for up to 28 days and 4 uses.

**Note: Caps must be placed on the correct vials after each use. Switching caps for re-calibrators and controls can negatively impact results.**

### Sample Collection and Storage

Extracted stool samples are appropriate for use in this assay.

Stool samples may be transported refrigerated. Samples should be received by the laboratory and analyzed within 72 hours of collection. If this is not possible, raw stool samples may be frozen at ≤ -80°C.

Stool extracts may be stored for up to 24 hours at room temperature, up to 6 days at 2-8°C or aliquoted and frozen at ≤ -80°C for up to four months.

## Reagent Preparation

All reagents, re-calibrators, and controls are ready to use. Bring all reagents and samples to room temperature (18 - 28°C) before loading on the KleeYa platform. Mix re-calibrators and controls by gentle inversion and load in appropriate KleeYa sample racks. Ensure no bubbles are present after mixing. If necessary, assign the lot specific assay to the appropriate control(s) on the KleeYa instrument.

## Sample Preparation: Stool Extraction and Dilution

Stool samples can be extracted by using:

- A. The Manual Weighing Procedure for Solid Samples
- B. The Liquid Sample Procedure
- C. The ALPCO Extraction Device Procedure

### A) The Manual Weighing for Solid Samples Procedure:

- A1.** Label and weigh (tare) an empty polypropylene tube together with the inoculation loop.
- A2.** Take out 20 to 100 mg of the stool sample by means of the inoculation loop and place it into the pre-weighed tube.
- A3.** Estimate the net amount of sample, break off the inoculation loop and leave the lower part of the loop in the tube.
- A4.** Add 1 x working Extraction Buffer to achieve 101X (for 20mg of stool, add 2mL of 1 x working Extraction Buffer) to the tube and close the tube.
- A5.** Proceed to Section D.

### B) The Liquid Sample Procedure

- B1.** Gently mix the stool sample
- B3.** Collect 50 µL
- B4.** Dispense into a clean, labeled polypropylene tube
- B5.** Add 5mL of 1X working Extraction Buffer to achieve 101X and close the tube.
- B6:** Proceed to Section D

### C) The ALPCO Extraction Device Procedure:

- C1 Refer to the detailed instructions provided with the EASY 2 Stool Extraction Device (80-EZEX2-100).
- C2. Proceed to Section D

**Note:** The extraction device should not be used for liquid stool samples. Liquid stool samples should be processed using the Liquid Sample Procedure

### D) Final Stool Processing for Manual and Device Procedures:

- D1.** Homogenize the extract samples on a multi-tube vortex by vigorous shaking (at highest speed) for 30 minutes.
- D2.** Centrifuge the extract in the tube for 3 minutes at 3,000 x g.
- D3.** Extraction devices can be loaded directly into KleeYa sample racks equipped with black spacers or can be decanted into a fresh labeled tube and tested on the KleeYa instrument or stored for future testing (See Sample Collection and Storage section).

The extraction procedure results in a 1:100 dilution. Samples are now ready for loading onto the KleeYa instrument. Contact ALPCO technical support for information on the correct sample rack and spacer to use with your tube type.

### Assay Procedure

1. Tap on the Load button in the main menu bar to show the Load screen.
2. Tap on the Samples button below the Load button.
3. Load all re-calibrator, control, and sample tubes into sample rack(s).
4. Load the sample rack(s), one after the other, into the sample loading bay
5. If necessary, assign desired tests and dilutions to the samples.
6. Tap on the Reagents button below the Load button.
7. Load all necessary Reagent Cartridges into reagent rack(s).
8. Load the reagent rack(s), one after the other, into the reagent loading bay
9. Load cuvettes, tips, and solutions and empty waste as required to complete the run.
10. Tap on the Start button to start the test run.

NOTE: Any additional information can be found in the KleeYa user Manual.

### Assay Calibration and Quality Control

1. The cut-off re-calibrator must be run each day *C. difficile* GDH testing is performed.
2. Positive and negative controls are to be run each day *C. difficile* GDH testing is performed.
3. Traceability: No international reference material or reference measurement procedures are available for *C. difficile* GDH. The Calibrators and Controls used in the Clostridium difficile GDH\_CLIA Kit are traceable to internal reference standards made from heat inactivated native *C. difficile* diluted in a buffer containing stabilizers and preservatives.

### Calculation of Results

The *C. difficile* GDH CLIA Kit results are directly analyzed in the KleeYa software. *C. difficile* GDH levels are expressed as arbitrary unit (AU) values and reported as a positive or negative result.

Results should be interpreted as follows:

AU	Result	Interpretation
$\leq 0.999$	Negative	Indicates the absence of <i>C. difficile</i> GDH
$\geq 1.000$	Positive	Indicates the presence of detectable <i>C. difficile</i> GDH

### Ancillary Devices

Both the pre-filled Easy 2 Stool Extraction Device (Product# 80-EZEX2-100) and the Extraction Buffer, 4X (Product# 10-EXBUF-225) for manual stool processing have been validated for use with the *C. difficile* GDH CLIA Kit. Please contact ALPCO regarding the compatibility of additional extraction devices and buffers.

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