MOLECULAR LAB PARTNERS

PRELIMINARY DOCUMENT – FOR INFORMATIONAL PURPOSES ONLY

Gastrointestinal Pathogen Panel 96 Well

Product Specification Sheet

RUO Product*

Product Description:

The Gastrointestinal (GI) Pathogen Panel is a multiplex polymerase chain reaction (PCR) assay for the identification of nucleic acids from 16 organisms frequently associated with GI tract infections. This method is highly accurate and analytically sensitive It is used to identify organisms by amplifying and detecting genetic material of pathogens in samples. The panel will aid in the research of causative agents of GI tract infections and their prevalence.

The target organisms included in the panel are as follows:
C. difficile: *C. difficile tcdA*, *C. difficile tcdB*
E. coli and *Shigella spp.*: Enterotoxigenic *E. coli* (ETEC), Shiga-like toxin producing *E. coli* (STEC), *Shigella*/Entroinvasive *E. coli* (EIEC)
Parasites: *Cryptosporidium spp.*, *Entamoeba histolytica*, and *Giardia lamblia*
Other GI Bacteria: *Campylobacter spp.*, *Salmonella spp.*, *Yersinia enterocolitica*, *Vibrio spp.*
Viruses: Adenovirus F40/F41, Norovirus GI/GII, Rotavirus A, Astrovirus

Product Information	
Gastrointestinal Pathogen Panel (96 well Plate)	
Part Number	P-XXX096-001-A
Number of Panels	12
Storage Temperature	-25°C to -15°C

Product Specifications	
QC Test	PCR Cycle Threshold Percent CV
Specification	TBD

QC Results	
Positive	TBD
Negative	TBD
Targets	TBD

Disclaimer - Use of PCR and Patent

This product is for basic PCR and is outside of any valid US patents assigned to Hoffman La-Roche.

* Limitations of Use

For Research Use Only. Not for use in diagnostic procedures.

Product Guarantee

This kit has been shown to generate reliable, repeatable and high-performance results. Please contact Molecular Designs for technical assistance. If not completely satisfied, our team will help you identify and address the issue and replace the assays as needed.

Jaspreet Seth

Jaspreet Seth
Vice President, Quality
Molecular Designs, LLC

Usage Information

Reagent Storage and Use Guidelines

1. Store all reagents at -25°C to -15°C.
2. Do not freeze-thaw plates more than 3 times.

The Following is Included in the Kit:

1. 96 well PCR plate pre-loaded with the Gastrointestinal Pathogen Panel assays and positive and negative controls.

The Following is Supplied by the User: Materials

1. Extracted Sample(s)
2. PCR optical film
3. Sealer for optical film

Equipment

1. Manual defrost -20°C freezer
2. Laminar Flow or PCR Dead Air Box for general plate setup. Do not use Laminar Flow for infectious samples
3. Pipette and appropriate filtered pipette tips
4. Plate Vortex [recommend Vortex Genie 2 (Model G560) with the 3-inch platform and rubber cover]
5. Plate centrifuge
6. Lab utility knife

Instrumentation

1. CFX96 Touch Real-Time PCR Detection System (or equivalent)

General Guidelines and Safety Precautions

1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.
   a) Do not pipette by mouth.
   b) Do not eat, drink, or smoke in designated work areas.
   c) Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples to prevent contamination. Avoid contaminating gloves when handling samples and controls.
   d) Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
   e) Thoroughly clean and disinfect all laboratory work surfaces.

   NOTE: Do not use sodium hypochlorite solution (bleach) to clean up a spill or to disinfect a plate before disposal as it can react with the common extraction reagents and generate toxic byproducts.

   If spills occur, follow internal procedures to immediately clean and decontaminate the surface of instrument.
2. A laminar flow or PCR Dead Air Box is recommended to reduce contamination probability.
3. The use of filtered, sterile and nuclease-free pipette tips is recommended.
4. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

12086 Miramar Pkwy
Miramar Florida 33025
www.prolabcorp.com
+1 305 600 0444

Usage Information

Reaction Plate Setup

1. Remove a reaction plate from the -20°C manual defrost freezer.
2. Determine the number of panels that will be used from the reaction plate. Score the foil seal on the PCR break-away plate using the lab utility knife and tear the plate along the perforated edge between samples wells to obtain the number of panels needed. Ensure the excess panels on the plate are properly labelled and sealed and promptly place the remainder back in the -20°C freezer.
3. Use the plate within 1 hour of thawing, keep sealed and store refrigerated at 4°C if not using immediately.
4. Spin down the plate for 30 seconds in a plate centrifuge.
5. Carefully remove the foil seal from the plate.
6. Add 4.0 µL of the sample being tested to each of the target wells.
7. Do not add any additional liquid to the Positive Control and Negative Control wells. All components have been added to these wells.
8. Seal the PCR plate using optical qPCR film. Note: If using a partial plate, remove the excess optical seal using the utility knife and ensure the plate is sealed.
9. Spin down the plate in a plate centrifuge.

Procedural Notes

1. Do not reuse consumables. They are for one-time use only.
2. Always use caution when transferring specimens from a primary collection tube to a secondary tube.
3. Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
4. Always use a new pipette tip for each specimen.
5. For testing of previously frozen sample, ensure samples are equilibrated to room temperature and well mixed prior to use.

Plate Layout (96 well plate, 12 panels, panel layout shown below) Not final - Subject to change

C. difficile tcdA / tcdB
Enterotoxin *E. coli* (ETEC) / Shiga-like toxin producing *E. coli* (STEC) / *Shigella spp.* / Enteroinvasive *E. coli* (EIEC)
Cryptosporidium spp. / *E. histolytica* / *G. lamblia*
Campylobacter spp. / *Salmonella spp.* / *Y. enterocolitica.* / *Vibrio spp.*
Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus
Endogenous Control
Positive Control
Negative Control

12086 Miramar Pkwy
Miramar Florida 33025
www.prolabcorp.com
+1 305 600 0444

Usage Information

Real-Time PCR Detection System PCR Run Setup

1. Open the specified run template and fill in the sample name fields with unique sample IDs corresponding to the samples being processed.
2. **NOTE:** This step can also be done prior to reaction plate setup if sample IDs have already been specified.
3. Place the reaction plate into the instrument in the appropriate orientation (A1 in the upper left corner), close the instrument lid and initiate the run.
4. **NOTE**: When running a partial plate, a balance is required at the other side of the instrument to ensure that the lid is sealed properly and doesn't break the instrument.

Amplification Interpretation and Troubleshooting

1. The laboratory should establish cycle threshold (CT) cutoffs as appropriate for their sample workflow and procedures. It is recommended that CT cutoffs are determined during the validation of the test.
2. The laboratory should evaluate the curve shape when considering whether a sample with a given CT should be considered positive:
   a) Plate sealing issues can lead to jagged curve shapes or rising/decreasing baselines that lead to inaccurate data (erroneous CT value).
   b) Inappropriate mixing or centrifuging can lead to inaccurate data.
3. If user suspects contamination, it is recommended to clean and disinfect the laboratory area and re-test to ensure proper results.
4. Any failure of the positive or negative control should require a repeat run. If the control failure continues, it is recommended to have the qPCR instrument and the sample extraction workflow evaluated to ensure they are functioning properly.

12086 Miramar Pkwy
Miramar Florida 33025
www.prolabcorp.com
+1 305 600 0444

GI 96 Well Plate Map – Not Final – Subject to change

	1	2	3	4	5	6	7	8	9	10	11	12
A	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*	*C. difficile tcdA / tcdB*
B	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*	Enterotoxin *E. coli* / Shiga-like toxin producing *E. coli* / *Shigella spp.*/ Enteroinvasive *E. coli*
C	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*	*Cryptosporidium spp. / E. Histolytica / G. lamblia*
D	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*	*Campylobacter spp. / Salmonella spp. / Y. enterocolitica / Vibrio spp.*
E	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus	Adenovirus F40/F41 / Norovirus GI/GII / Rotavirus A / Astrovirus
F	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control	Endogenous Control
G	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control
H	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control

12086 Miramar Pkwy
Miramar Florida 33025
www.prolabcorp.com
+1 305 600 0444