

## Polymerase Chain Reaction (PCR) Kit for the Detection of *Blastocystis hominis*

**Product Name: BlastocystisFind™ Version 1.0**

**Catalog # BF001: 100 Reactions (50 µl each)**

## Technical Bulletin

## Introduction:

### BlastocystisFind™ Kit:

Background: *Blastocystis* infections are prevalent in the human population and in particular in AIDS patients and other immunosuppressed patients. Symptoms include abdominal pain, diarrhea, constipation, vomiting and fatigue. *Blastocystis hominis* is a species of parasitic protozoa found in the intestines of humans and other primates. It was classified as a yeast in 1912. This organism is now recognized as a protozoan parasite of humans causing intestinal disease with potentially disabling symptoms.

### Kit components:

The kit provides all the necessary ingredients to perform a PCR reaction except for Taq Polymerase. The primer mix and Buffer/dNTP solutions are shipped in separate tubes to ensure a longer kit shelf life. For best results, all reagents included in the kit should be kept frozen at  $-20^{\circ}\text{C}$  and the user should avoid repeated freeze-thaw cycles.

1. Buffer/dNTP 1x mixture
2. Multiplex primer mix (5X)
3. One tube of Positive Control
4. One tube of Molecular Biology Grade Water

### Reagent Preparation:

For preparation of the mastermix, add 300  $\mu\text{l}$  of the primer mix to a tube of Buffer/dNTP mix. Mix by vortexing for 5 seconds. Add the template DNA and Taq Polymerase to the reaction mixture, and start cycling on a certified thermal cycle.

### Reaction Mixture:

Template DNA	10 $\mu\text{l}$
Premixed reaction mixture	40 $\mu\text{l}$
Taq Polymerase	0.15 $\mu\text{l}$

Mix well by pipetting up and down 4 times and start cycling.

### Cycling Conditions:

Temperature	Time	Cycles
94° C	3 minutes	1 X
94° C	30 seconds	35X
58° C	30 seconds	
72° C	35 seconds	
72° C	2 minutes	1X
4° C	Forever	

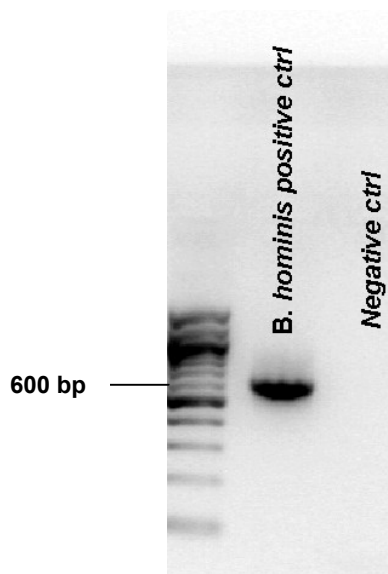
These conditions have been validated on a Bio-Rad Gene Cycler, and a Stratagene Gradient 96 Robocycler.

### Technical tips:

1. It is recommended to use a DNA extraction methodology that is proven to yield high quality DNA and that is manufactured under GMP conditions.
2. Use clean disposable gloves when performing the assay and make sure that the work area is clean prior to starting the assay setup.
3. Keep your reagents and PCR mixture tubes on a cold block during reaction setup.
4. Use positive displacement pipettes.

5. Avoid splashes and if splashing occurs, change gloves.
6. Add DNA last and cap each tube before proceeding to the next tube.
7. The amplification and detection areas should be physically distant (do not use the same bench area to setup the PCR reactions and run your gels. PCR product contamination can cause serious problems.

The gel image below is a sample of what a typical reaction using the recommended cycling conditions should look like:



### Troubleshooting:

1. No signal in positive control lane: annealing temperature too high. Use recommended annealing temperature and make sure that your cycler is calibrated and the temperature on the display is the actual block temperature.
2. Too many bands: annealing temperature too low, increase annealing temperature gradually. This could also be due to PCR mis-priming prior to cycling. Make sure your PCR reaction tubes are kept cool to avoid priming before cycling. The initial cycles are critical. Alternatively, use a hotstart Taq Polymerase.
3. Negative control shows a PCR product: this is due to contamination of either the mastermix, the water template used in the negative control tube or the pipette tip used to mix the negative control reaction mixture.

### References:

Scicluna, S.M., Tawari, B., Clark, C.G. DNA barcoding of *Blastocystis*. *Protist*. 2006, Feb; 157(1): 77-85. Epub 2006, Jan 23.