# Group B *Streptococcus* Detection Protocol

## A. Introduction

This protocol uses end point PCR† with TaqMan® probes to test for Group B *Streptococcus* (GBS). The target sequence is the surface immunogenic (*sip)* gene, which is specific to GBS.

PCR run time is 38 min.

## B. Equipment

- Spartan DX™ instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

## C. Materials

- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- Filtered pipette tips
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- BIOTUB-QT kit (Biotools, Cat. No. 90.572C)
- Sterile water (DNase-free)
- 1.5 ml microcentrifuge tubes
- Primers that recognize the *sip* gene (Integrated DNA Technologies)(Table 1)
- TaqMan probe (Biosearch Technologies) that recognizes the *sip* gene (Table 1)

| Primer/Probe | Forward (5'-3') | Reverse (5'-3') | Amplicon size (bp) |
|---|---|---|---|
| *sip* primers | gAg CgT TCC ggT AgC ACA AAA | TgA ggT Tgg AgC CCT gCA TT | 108 |
| *sip* probe | 6-FAM - CCA ACA gCA ACA CCg gTA gCA CAA CCA- BHQ-1 | | |

6FAM = 6-carboxy-fluorescein, BHQ-1 = Black Hole Quencher 1

***Table 1.*** Primer/probe sequences and amplicon size.

## D. Preparation

1. Any commercially-available DNA purification kit may be used
2. Turn on Spartan DX instrument and let it warm up for a minimum of 10 min
3. Set up thermal cycling program as per Table 2

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95.0°C | 10 s | 1 |
| Denaturation | 95.0°C | 25 s | 50 |
| Annealing/extension | 50.0°C | 20 s | 50 |

***Table 2.*** Cycling parameters.

**E. Protocol**

1. Prepare a master mix for *GBS* as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
   - Tube 1: 18 µl of GBS master mix
   - Tube 2: 18 µl of GBS master mix
   - Tube 3: 18 µl of GBS master mix
   - Tube 4: 18 µl of GBS master mix
3. In a separate lab area, add the following to each tube:
   - Tube 1: 1 µl of purified DNA (sample in question)
   - Tube 2: 1 µl of known GBS-positive DNA + 1 µl of sterile water (positive control)
   - Tube 3: 1 µl of purified DNA (sample in question) + 1 µl of known GBS-positive DNA (spiked control)
   - Tube 4: 2 µl of sterile water (negative control)
4. Mix and spin down reactions
5. Overlay reaction mixture with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert samples into Spartan DX instrument and start your run
8. End point PCR is determined to be positive if the last cycle reading has a fluorescence value greater that 5
9. Example of real-time graph is depicted in Figure 1

| Reagent | GBS Master Mix | |
|---|---|---|
| | **Reaction Formulation** | **Volume** |
| QUANTIPROBES Reaction Mix (2X) | 10 µl x (_____+0.5*) Sample # | |
| Forward primer (10 µM) | 0.4 µl x (_____+0.5*) Sample # | |
| Reverse primer (10 µM) | 0.4 µl x (_____+0.5*) Sample # | |
| Probe (2 µM) | 2 µl x (_____+0.5*) Sample # | |
| Sterile water | 5.2 µl x (_____+0.5*) Sample # | |
| **Total volume of master mix** | **18 µl/reaction** | **____ µl** |

* Recommended volume correction factor for pipetting error.

***Table 3.*** Components of PCR master mix for GBS.

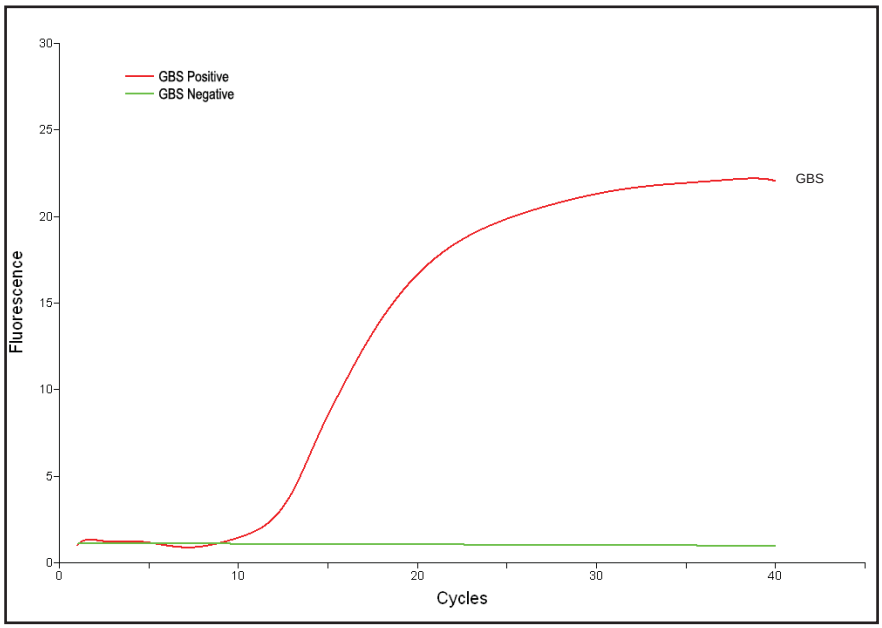


***Figure 1.*** Real-time PCR result for Group B *Streptococcus* (GBS).

† - An end point assay is described as an assay that uses data from images collected at the first and last cycles of a PCR run to determine the success or failure of the reaction. End point analysis mode is selected in the options menu of the SpartanDX™.