Cat. # CY234

For Research Use

TaKaRa

# CycleavePCR™ GBS Capsular Typing Kit

Product Manual

v201903Da

# Table of Contents

## I. Description

*Streptococcus agalactiae* (also known as Group B hemolytic *Streptococcus* or GBS) is a gram-positive bacteria that is part of the normal flora in the human genitourinary and gastrointestinal tracts. GBS can cause opportunistic infections, including meningitis in the elderly and sepsis immediately after birth in newborns.

The type of capsular polysaccharides produced by bacteria play an important role in the establishment of infection. For GBS, there are 10 known capsular types. Types Ia, Ib, and III have been associated with sever sepsis and meningitis in newborns. Therefore, rapid and accurate typing of GBS is important.

The CycleavePCR GBS Capsular Typing Kit is designed for detection of the *cps* gene that encodes the capsular type and can be used to identify GBS capsular types Ia, Ib, and III. This kit can be used in conjunction with the CycleavePCR *Streptococcus agalactiae* (GBS) Detection Kit (Cat. #CY233), a kit that tests for the presence of GBS.

This kit enables amplification products to be detected by cycling probe technology, which provides highly sensitive detection through the combined use of a RNA/DNA chimeric probe and RNase H. This enables efficient detection of specific sequences of the gene fragment during and after amplification. One end of the probe is labeled with a fluorescent moiety and the other end with a quencher. When intact, this probe does not emit fluorescence, due to the action of the quencher. However, when it forms a hybrid with the complementary sequence of an amplification product, RNase H cleaves RNA in the chimeric probe, resulting in strong fluorescent signal emission (see Figure 1). The amount of amplified product can be monitored by measuring the intensity of emitted fluorescence.

This kit contains FAM-labeled probes for detecting the *cps* (Ia) and *cps* (III) genes, and a ROX-labeled probe for detecting *cps* (Ib). By simultaneously monitoring two wavelengths, *cps* gene detection and typing can be achieved. The kit relies on real-time PCR detection, which requires no electrophoresis and yields results quickly.

This kit includes *TaKaRa Ex Taq*® HS, a hot-start PCR enzyme, which prevents non-specific amplifications caused by mispriming or primer dimer formation during reaction mixture preparation or other pre-cycling steps. The use of *TaKaRa Ex Taq* HS therefore allows high-sensitivity detection.

This kit was developed under the supervision of Dr. Kimiko Ubukata, Department of Infectious Diseases, Keio University School of Medicine.

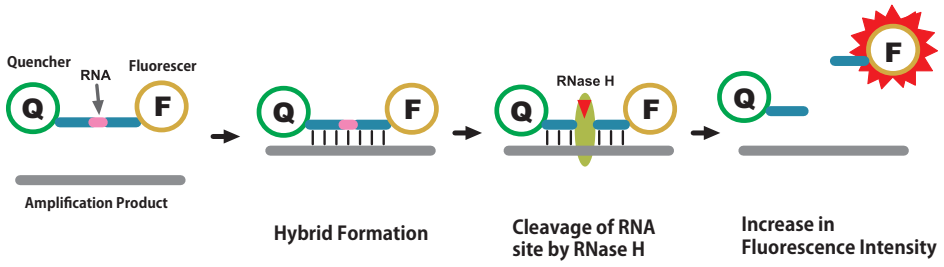



Figure 1. Principle of Cycling Probe Technology.

## II. Components (50 reactions, 25 μl volume)

| | | | |
|---|---|---|---|
| 1. | 2X CycleavePCR Reaction Mixture | 625 μl | |
| 2. | cps (Ia) & cps (Ib) Primer/Probe Mix (FAM, ROX) | 50 μl | ＊1 |
| 3. | cps (III) Primer/Probe Mix (FAM) | 50 μl | ＊1 |
| 4. | $dH_2O$ | 500 μl | |
| 5. | cps (Ia) Positive Control | 30 μl (15 rxns) | ＊2 |
| 6. | cps (Ib) Positive Control | 30 μl (15 rxns) | ＊2 |
| 7. | cps (III) Positive Control | 30 μl (15 rxns) | ＊2 |

＊1 Contains a fluorescent-labeled probe; store protected from light.
＊2 To avoid contamination, store separately from components 1 - 4.

[Component Information]

**2X Cycleave Reaction Mixture**:
A PCR reagent containing enzymes, buffer, and a dNTP mixture.

**cps (Ia) & cps (Ib) Primer/Probe Mix (FAM, ROX) and cps (III) Primer/Probe Mix (FAM):**
Mixtures containing primers and probes for detecting the *cps* (Ia), *cps* (Ib), and *cps* (III) genes.
The *cps* genes (the target genes) are amplified with the appropriate primers. The FAM-labeled and ROX-labeled probes detect the *cps* genes.

**Target genes:** *cps* genes encoding the GBS capsular type Ia, Ib, or III.

**cps (Ia) Positive Control:** Positive control DNA for the *cps* (Ia) gene.

**cps (Ib) Positive Control:** Positive control DNA for the *cps* (Ib) gene.

**cps (III) Positive Control:** Positive control DNA for the *cps* (III) gene.

## III. Storage

-20℃

## IV. Materials Required but not Provided

[Real-time PCR]

- Real-time PCR instrument and tubes
  Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)＊
  Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)＊
  Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific), etc.
- Benchtop centrifuge
- 1,000-μl, 200-μl, 20-μl, and 10-μl micropipettes
- Micropipette tips (with hydrophobic filter)

＊ Not available in all geographic locations. Check for availability in your area.

## V. Precautions

1) Operate real-time PCR instruments in accordance with the manufacturer's instructions.

2) The chimeric probe and primers are susceptible to degradation by nucleases, and if degraded, cannot provide accurate detection. Take care to avoid nuclease contamination from sources such as perspiration or saliva introduced during sample handling.

3) It is recommended to designate and physically segregate the 3 areas described below for the processes from preparation of reaction mixtures to detection. Avoid opening/closing tubes containing amplification products in any of these areas:
   - ○ Area 1: reaction mixture preparation and dispensing
   - ○ Area 2: sample preparation
   - ○ Area 3: addition of samples to reaction mixtures, reaction, and detection

   This kit allows amplification and detection to take place simultaneously in real time. Thus, no electrophoresis or other analytical methods are required after the reaction is complete. Never remove amplification products from tubes, as doing so may introduce contamination.

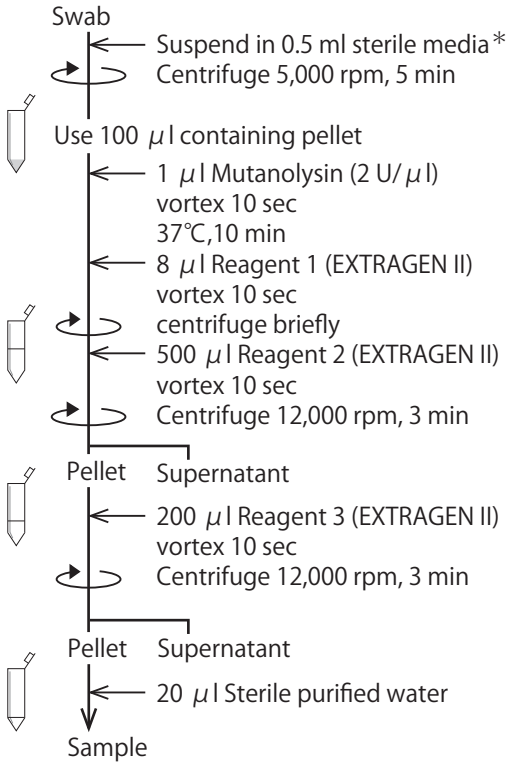
4) Results obtained with this kit are analyzed using a real-time PCR amplification instrument. Failure of any of the auto functions on the real-time PCR amplification instrument may lead to erroneous analysis of results. Make sure that the settings on the real-time PCR amplification instrument are adjusted correctly, as described in the instrument manual.

## VI. Protocol

Overview

1. Sample Preparation (Work in Area 2)

   Use the preparation method described in CycleavePCR *Streptococcus agalactiae* (GBS) Detection Kit (Cat. #CY233) User Manual




2. Real-time PCR instrument setup
3. Reaction mixture preparation and reaction start

   Prepare the reaction mixture and dispense in tubes (Work in Area 1)

| Reagent | Volume |
|---|---|
| 2X CycleavePCR Reaction Mixture 🟠 | 12.5 µl |
| Primer/Probe Mix (FAM, ROX) 🟤 or 🟡 | 2.0 µl |
| sample DNA or Positive Control or $dH_2O$ | 2.0 µl |
| $dH_2O$ ○ | 8.5 µl |
| Total | 25 µl |

   Add the negative control ($dH_2O$) (in Area 1), and the samples and positive control (in Area 3).

   Set the tubes in the real-time PCR instrument; start the reaction.

4. Results
5. Analysis of Results

＊ MUELLER HINTON BROTH, etc.

### VI-1. Sample Preparation (Work in Area 2)

Samples prepared using the procedure in the CycleavePCR *Streptococcus agalactiae* (GBS) Detection Kit (Cat. #CY233) can be used.

**Preparation example using EXTRAGEN II**

Prepare template DNA from the sample using a reagent such as EXTRAGEN II (Tosoh, Co., Ltd.).

(1) Sterily place the sample swab in 0.5 ml of sterile media and suspend. Centrifuge at 5,000 rpm for 5 min. Remove 400 μl of the supernatant.

(2) Add 1 μl Mutanolysin (2 U/μl)* to the remaining 100 μl sample and vortex briefly to mix. Incubate for 10 min at 37℃.

* Preparing Mutanolysin: Dissolve Mutanolysin (Lyophilized, Sigma-Aldrich, Cat. #M4782) in sterile water to obtain a final concentration of 2 U/μl. Aliquot the solution and store at -20℃.

(3) Add 8 μl Reagent 1 (co-precipitation reagent), vortex for 10 sec to mix, and centrifuge briefly.

(4) Add 500 μl Reagent 2 (propanol, protein denaturant) and vortex for 10 sec to mix.

(5) Centrifuge at 12,000 rpm for 3 min. Discard the supernatant. Centrifuge briefly again and remove any residual supernatant.

(6) Add 200 μl Reagent 3 (propanol, potassium chloride) and vortex for 10 sec to mix.

(7) Centrifuge at 12,000 rpm for 3 min. Discard the supernatant. Centrifuge briefly again and remove any residual supernatant.

(8) Dissolve the pellet in 20 μl of sterile purified water. Keep sample on ice. If the sample will not be used immediately, store at -20℃.

**Note**: Use filtered tips for all steps.

### VI-2. Preparation of Reaction Mixture

This kit allows the simultaneous detection of amplification products from both the *cps* (Ia) and *cps* (Ib) genes in a single tube when the cps (Ia) & cps (Ib) Primer/Probe Mix is used. Amplification products from the *cps* (III) gene are detected when the cps (III) Primer/Probe Mix is used. **To obtain accurate detection results, perform the positive control reaction and the negative control reaction for the *cps* gene simultaneously.**

＊ To maximize the reliability of your results, we recommend running at least 2 reactions per sample.

(1) Prepare the following reaction mixture on ice. (Work in Area 1)

Prepare two reaction solutions, one for each Primer/Probe Mix.

Prepare a premix containing components other than the sample template in volumes sufficient for the required number of tubes plus a few extra. Dispense aliquots of 23 μl and cap loosely. Set up one of the tubes as a negative control by adding 2 μl of sterile water and capping the tube tightly.

The number of tubes required for the cps (Ia) & cps (Ib) Primer/Probe Mix is defined as the number of samples +3 (one for the negative control reaction and one for each of the two positive control reactions).The required number of tubes for the cps (III) Primer/Probe Mix is defined as the number of samples + 2 (one for the negative control reaction and one for the positive control reaction).

| Reagent | Volume (1 rxn) | Final Conc. |
|---|---|---|
| 2X CycleavePCR Reaction Mixture 🟡 | 12.5 μl | 1X |
| Primer/Probe Mix (FAM, ROX) 🟤 or 🟡 | 2.0 μl | 1X |
| Sample, Positive Control＊1, or $dH_2O$ | (2.0 μl)＊2 | |
| $dH_2O$ ○ | 8.5 μl | |
| Total | 25 μl | |

＊1 For the positive control reaction, use both the cps (Ia) Positive Control and the cps (Ib) Positive Control in the reaction with the cps (Ia) & cps (Ib) Primer/Probe Mix; use the cps (III) Positive Control in the reaction with the cps (III) Primer/Probe Mix.

<u>For the cps (Ia) & cps (Ib) Primer/Probe Mix</u>
Positive Control Reaction 1: cps (Ia) Positive Control 🔴
Positive Control Reaction 2: cps (Ib) Positive Control 🩷

<u>For the cps (III) Primer/Probe Mix</u>
Positive Control Reaction1: cps (III) Positive Control 🟡

＊2 Add the sample or the Positive Control DNA in step (2), not in this step.

(Move to Area 3)

(2) Add sample (template). (Work in Area 3)
Add either the sample or the positive control to all tubes except the negative control tube and then cap tightly. Be sure to wear gloves.

Briefly centrifuge the tubes in a table top centrifuge and then place them in a real-time PCR instrument.

[Precaution]
Start reactions within 1 hour of preparing the reaction mixtures.

### VI-3. Amplification and Detection (Work in Area 3)

Operating procedures differ depending on the real-time PCR instrument used. For specific operating procedures, refer to the instrument manual.

**[For the Thermal Cycler Dice Real Time System //]**

PCR Conditions
- Initial denaturation (Hold)
  - Cycle: 1
  - 95℃ 2 min
- 3-step PCR
  - Cycles: 40
  - 95℃ 10 sec
  - 50℃ 30 sec
  - 72℃ 20 sec (detection)

Detection Filter
- FAM
- ROX

Sample layout

Internal Control Detection Filter
- none

Sample Types
- Negative control
  - Sample Type: NC
- Positive Control
  - Sample Type: PC
- Test Sample
  - Sample Type: UNKN (unknown)

Target type
- cps (Ia) /cps (Ib) [for the cps (Ia) & cps (Ib) Primer/Probe Mix Reaction]
  - Target: A
- cps (III) [for the cps (III) Primer/Probe Mix Reaction]
  - Target: B

**[ For the Applied Biosystems 7500 Fast Real-Time PCR System, StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)]**

Use Quantification-Standard Curve mode. To change the default settings, use Advanced Setup.

PCR Conditions

Initial Denaturation (Hold)
Cycle: 1
95℃ 2 min

3-step PCR
Cycle: 40
95℃ 10 sec
50℃ 30 sec
72℃ 25 sec＊(detection)

＊ Time varies depending on the real-time PCR device used. Refer to the instrument manual for reaction conditions.

Passive Reference

none

Define Targets

Target Name: cps (Ia), Reporter: FAM, Quencher: (none)
Target Name: cps (Ib), Reporter: ROX, Quencher: (none)
Target Name: cps (III), Reporter: FAM, Quencher: (none)

Define Samples

Negative Control
Sample Type: NTC (No Template Control)
Positive Control
Sample Type: Standard or Unknown
Test Sample
Sample Type: Unknown

**Note:** The same procedure can be used for the StepOnePlus Real-time PCR System. However, since the detection sensitivity for ROX is low, the ROX (*cps* (Ib)) amplification curve will appear small when all targets are displayed simultaneously. In order to analyze the ROX and FAM curves, display them separately.

## VII. Analysis of Results

Analysis 1: Unknown samples (Final analysis should be based on the results of each control reaction)

[For the cps (Ia) & cps (Ib) Primer/Probe Mix Reactions]

| | Amplification signal | |
|---|---|---|
| FAM (*cps* (Ia) detection) | (+) | *cps* (Ia) gene positive |
| | (–) | *cps* (Ia) gene below the limit of detection |
| ROX (*cps* (Ib) detection) | (+) | *cps* (Ib) gene positive |
| | (–) | *cps* (Ib) gene below the limit of detection |

[For the cps (III) Primer/Probe Mix Reaction]

| | Amplification signal | |
|---|---|---|
| FAM (*cps* (Ia) detection) | (+) | *cps* (III) gene positive |
| | (–) | *cps* (III) gene below the limit of detection |

Analysis 2: Positive control

[For the cps (Ia) & cps (Ib) Primer/Probe Mix Reactions]

For the cps (Ia) Positive Control reaction, confirm that amplification is detectable using the FAM filter, but is absent for the ROX filter.

For the cps (Ib) Positive Control reaction, confirm that amplification is detectable using the ROX filter, but is absent for the FAM filter.

[For the cps (III) Primer/Probe Mix Reaction]

For the cps (III) Positive Control reaction, confirm that amplification is detectable using the FAM filter.

Analysis 3: Negative control

[For the cps (Ia) & cps (Ib) Primer/Probe Mix Reaction]

For the negative control reaction, confirm that amplification is not detectable using the FAM and ROX filters.

[For the cps (III) Primer/Probe Mix Reaction]

For the negative control reaction, confirm that amplification is not detectable using the FAM filter.

## VIII. Troubleshooting

- If the Ct is 33 cycles or higher, confirm specific amplification of target genes by agarose gel electrophoresis. When performing electrophoresis, be careful to avoid contamination of the PCR products.

  <Amplification product size>

  | | | | | |
  |---|---|---|---|---|
  | *cps* (Ia) | from samples: | 210 bp | from cps (Ia) Positive Control: | 168 bp |
  | *cps* (Ib) | from samples: | 194 bp | from cps (Ib) Positive Control: | 153 bp |
  | *cps* (III) | from samples: | 281 bp | from cps (III) Positive Control: | 216 bp |

- When an amplification curve is detected in the negative control reaction (NC).
  → Contamination may have occurred. Decontaminate the bench area used to prepare reaction mixtures as well as the apparatuses and instruments used. Then repeat the reaction.
- When the FAM filter or the ROX filter show no amplification curve in the positive control reaction (PC).
  → The PCR reaction or the cycling probe detection failed. Repeat the reaction.
  There may be a problem with the Primer/Probe mix or the positive control may have been degraded.
- When both the FAM filter and the ROX filter do not show an amplification curve for the cps (Ia) & cps (Ib) Primer/Probe Mix and the cps (III) Primer/Probe Mix in the sample reaction.
  → If the sample was determined to contain GBS using the CycleavePCR *Streptococcus agalactiae* (GBS) Detection Kit (Cat. #CY233), the capular type may be something other than type Ia, Ib, or III.

## IX. Related Products

CycleavePCR™ *Streptococcus agalactiae* (GBS) Detection (Cat. #CY233)
Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)*
Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)*
0.2 ml 8-strip tube, individual Flat Caps (Cat. #NJ600)
96 well Hi-Plate for Real Time (Cat. #NJ400)
Sealing Film for Real Time (Cat. #NJ500)
48 well snap plate (Cat. #NJ700)
Flat cap for snap plate (Cat. #NJ720)
Plate Sealing Pads (Cat. #9090)

* Not available in all geographic locations. Check for availability in your area.