Group B Streptococcus DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3056E

Product Name

Group B Streptococcus DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification

24 tests/kit, Pre-packaged 12 tests/kit

Intended Use

The Group B Streptococcus DNA Fluorescence Diagnostic Kit (PCR-Fluorescence Probing) is intended to detect Group B streptococcus in genital tract secretions and rectal secretions collected from 34-37 weeks pregnant women by applying real-time quantitative PCR technique. The detection results can be used as an aid in the diagnosis of Group B streptococcus infection, but can not be used for screening.

For in vitro diagnostic use only. For professional use only.

Summary

Group B Streptococcus (GBS) was named for that the structure of polysaccharides in cell wall belongs to group B in the classification of antigenic structure. GBS is an opportunistic pathogen, locating in digestive tract and urinary tract of human. The proportion of GBS carrying rate is up to 15%~35% in healthy person, and 10%~30% in pregnant women.

GBS could cause pregnant women genital tract infection, early-onset neonatal infections and late onset neonatal infections. In pregnant women infections, it may cause premature labour, fetal dysplasia, premature rupture of membranes and late abortion[1]. According to statistics, about 10%~30% of pregnant women are infected with GBS, and 40%~70% of them are likely to transmit to the next generation. In early-onset neonatal infections (within 7 days after birth), there are high risks of neonatal hematosepsis and pneumonia, which is one of the main causes of neonatal death[2]. While in the late onset neonatal infections, meningitis and severe neural sequela may occur, including hydrocephalus, mental retardation, microcephaly, deafness, etc.

Test Principle

By applying real-time fluorescence quantitative PCR technology, this diagnostic kit utilizes one pair of specific primers and a fluorescence probe which are designed to target GBS DNA conserved sequences, accompanied with other ingredients in GBS-PCR Mix, to achieve fast detection of GBS DNA through the changes of the fluorescent signals.

The PCR detection system uses UNG enzyme + dUTP contamination-proof system to fully degrade possible PCR amplified products in order to avoid a false positive result.

The PCR detection system uses a internal positive control for the evaluation of amplification reaction of specimens and monitor the presence of PCR inhibitors, as well as to evaluate the nucleic acid extraction efficiency, in order to avoid a false negative result.

Components of the kit

No.	Reagent Name	Specification & Qty.		Main Ingredients
		24T	Pre-packaged 12T	
1	GBS-Enzyme Mix	48 μL/tube × 1 tube	2 μL/tube × 12 tubes	DNA polymerase, Uracil-N-Glycosylase enzyme
2	GBS-PCR Mix	912 μL/tube × 1 tube	38 μL/tube × 12 tubes	Primers, probes, dNTPs, 10 x PCR buffer, sterile purified water
3	GBS-Negative Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Normal saline
4	GBS-Positive Control	1000 μL/tube × 1 tube	1000 μL/tube × 1 tube	Cloned plasmid containing target gene fragment of GBS
5	GBS-Internal Control	25 μL/tube × 1 tube	1 μL/tube × 12 tubes	Cloned plasmid containing target gene fragment of internal positive control

Note:

1. Do not mix or exchange components from different kits.
2. All biological materials within the detection kit have been inactivated.
3. Materials required but not provided: 1.5 mL DNase-free and RNase-free centrifuge tubes, 0.2 mL PCR reaction tubes, pipette tips (10 μL, 200 μL and 1000 μL tips with filters are preferred), desktop centrifuge, desktop vortex mixer, magnetic-bead separator, various models of pipettes.
4. Not included in the kit reagent: sterile saline, Nucleic Acid Extraction-Purification Kit (Reference Number: S50016E Series) or Sample Release Reagent (Reference Number: S1013E Series) manufactured by Sansure Biotech Inc.

Precautions

1. For *in vitro* diagnostic use only. Please read the product manual carefully before operation.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory; laboratory personnel must receive professional training; test processes must be performed in separated rooms; all consumables should be for single use only after sterilization; special instruments and devices should be used for every process; all lab devices used in different processes and rooms should not be cross-used.
4. All specimens for detection should be handled as if infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between specimens. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.
5. Before use, all reagents must be fully thawed at room temperature and mixed thoroughly.
6. After the addition of the sample in the tube the resulting solution is to be considered potentially biohazardous, handle the reagent with appropriate precautions and good laboratory practice.
7. The safe disposal of the reagents supplied must be carried out according to the instruction contained in the specific Safety Data Sheets and in compliance with the national regulations on disposal of potentially hazardous waste.
8. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established; If you have any questions about the test or the results, please contact Sansure`s customer service hotline +86-731-88883176-6116 or send an email to info@sansure.com.cn/ support@sansure.com.cn.

Storage and Validity

The shelf life of the kit is 12 months at -25°C to -15°C and protected from light. Please see the manufacturing date and expiry date on the outer package.

1. The reagents keep valid and stable within the expiry date if not used. As long as the container of the reagent is opened, the freeze/thaw cycles of amplification reagent should not exceed five.
2. The reagents keep valid within 7 days during the shipment in a sealed foam box with ice.

Compatible Instrument

The kit is compatible to Fluorescence Quantitative Analysis System containing FAM, HEX/VIC channels:

1. Applied Biosystems/7500 Real-Time PCR System
2. Hongshi/SLAN®-96P Real Time PCR System
3. Molarray/Real-time Quantitative Thermal Cycler (Model: MA-6000)
4. Sansure/Portable Molecular Diagnostic System (S-Q37A/S-Q37B)
5. Sansure/Portable Molecular Workstation (Model: S-Q36A)
6. Sansure/Portable Molecule Workstation (Model:S-Q31A/S-Q31B)

Specimen Requirements

1. Applicable specimen type: genital tract secretions and rectal secretions swab.
2. Specimen collection: (The objects are required not to urinate in 2 hours before specimen collection)

Clear the excessive secretion in genital tract, and use a cotton swab to collect the specimens. Rotate the swab gently 1-2 circles around the genital tract when the swab locates at 1/3 of the lower genital tract. Then insert the swab to anus and touch 2-5 cm upstream of sphincter, rotate gently 1-2 circles to collect specimen. At last, place the swab with specimen in a sterile specimen collection tube, and seal and send it for detection.

3. Specimen storage and delivery: Specimens collected via the above-mentioned method can be used for immediate detection, or stored at 2-8°C for up to 24 hours, or at -20°C for up to 7 months. Freeze/thaw cycles should not exceed seven. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice.

Test Method

1. Please process according to the following steps for 7500 Real-Time PCR System, SLAN®-96P Real Time PCR System, Real-time Quantitative Thermal Cycler (Model: MA-6000) instruments:

1.1 Pretreatment of specimens (performed at "specimen processing room") (GBS-negative control, GBS-positive control and specimens are processed together.)

1.1.1. Add 1 mL of sterile saline to the specimen collection tube, and vortex it thoroughly to wash the cotton swab, and then press to dry the cotton swab against the tube wall and discard it.

1.1.2. Pipette 500 μL of sample from the sample collection tube into a 1.5 mL centrifuge tube as the test sample. Refer to the following steps to process the samples.

Method 1: It is recommended to use Sample Release Reagent (Reference Number: S1013E Series) manufactured by Sansure Biotech Inc to extract the nucleic acid as per its instructions.

Method 2: It is recommended to use Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number: S50016E Series) manufactured by Sansure Biotech Inc to extract the nucleic acid as per their instructions.

Remarks: The specimens processed via the above steps can be used to test GBS-DNA.

1.2. Preparation of reagent (performed at "reagent preparation room")

1.2.1 Take all of the components out of the detection kit and equilibrate them to room temperature. When the components temperature has reached room temperature, mix well for future use.

1.2.2 According to the quantity of pretreated specimens, GBS-Negative Control and GBS-Positive Control, pipette appropriate quantity of GBS-PCR Mix, GBS-Enzyme Mix and GBS-Internal Control (GBS-PCR Mix 38 μL/test+ GBS-Enzyme Mix 2 μL/test + GBS-Internal Control 1 μL/test), fully mix them to make a PCR-Mastermix and then centrifuge it instantaneously for later use.

	1 specimen	10 specimens	24 specimens
GBS-PCR Mix (μL)	38	380	912
GBS-Enzyme Mix (μL)	2	20	48
GBS-Internal Control (μL)	1	10	24

Note: The above configuration is just for your reference and to ensure enough volume of the PCR-Mastermix, more volume of the actual pipetting may be required.

1.3. Loading (performed at "specimen processing room")

1.3.1 Add 10 μL of pretreated GBS-Negative Control, GBS-Positive Control and specimens respectively into each PCR reaction tube (pipette the liquids up and down and then absorb).

1.3.2 Add 40 μL of PCR-Mastermix to each tube and cap it (flick the tube to remove bubbles if exist), then centrifuge it at 2000 rpm for 30 seconds.

1.4. PCR Amplification (performed at "amplification and analysis room") (refer to user manual of each instrument to adjust the settings)

1.4.1 Place the PCR reaction tube into the sample well of the amplification device. Set up the GBS-Negative Control, GBS-Positive Control and unknown specimens in the corresponding sequence and input specimen information.

1.4.2 Select PCR test channel:

a. Select FAM channel (Reporter: FAM, Quencher: None) to test GBS-DNA.

b. Select HEX or VIC channel (Reporter: HEX/VIC, Quencher: None) to test GBS-Internal Control.

c. Set Passive Reference: None.

d. Set Sample Volume: 50 μL.

1.4.3 Set cycle parameters:

	Step	Temperature	Time	Cycle No.
1	UNG enzyme reaction	50°C	2 min.	1
2	Taq enzyme activation	94°C	5 min.	1
3	Denaturation	94°C	15 sec.	45
	Annealing, extension, fluorescence collection	57°C	30 sec.*	
4	Device cooling (optional)	25°C	10 sec.	1

Note: Due to the technical specification of 7500 Real-Time PCR System, it can not be set at 30 sec. but 31 sec. or 32 sec.

2. Please process according to the following steps for Portable Molecule Workstation (Model: S-Q31A/ S-Q31B):

2.1 Preparation of consumables and reagents

(The well location information has been marked on the supporting consumables)

(1) Take out the reaction tube carrier, PCR reaction tube and Tip.

(2) Put the **Tip** into **Well H**,and PCR reaction tube into **Well PCR** (The well location information has been marked on the reaction tube carrier)

(3) Put internal control into the **Well A**; Put Sample Release Reagent into the **Well B**; Put GBS-PCR Mix into the **Well C**; Put GBS-Enzyme Mix into the **Well D**.

(4) Add 20μL sample to be tested or GBS - Positive Control or GBS - Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

2.2 Test Procedure (Refer to user manual of each instrument to adjust the settings.)

2.2.1 Gently press the front door to open it.

2.2.2 Place the **Well A** of reagent strip into the instrument towards the outside of the instrument, and close the front door of the instrument.

2.2.3 Click the "**Lab task**" on the instrument display screen to enter the interface of setting new experimental task.

2.2.4 Select the required experimental project in the drop-down menu of **Lab project**, enter the corresponding task name in the **Task Name** bar, and input and select other items that should be input or selected.

2.2.5 Click **"Submit"** to submit the experimental task and **"OK"** to run the instrument and start the experimental task successively.

2.2.6 When the Portable Molecule Workstation (Model: S-Q31B) shows **"Please transfer the PCR tube to the 1/2/3/4"**(The S-Q31A shows "Please transfer the PCR tube") on the interface, take out the PCR tube and cover it well, then centrifuge it instantaneously.

2.2.7 Insert the PCR tube into the PCR amplification module (the "PCR 1/2/3/4" cover has been automatically opened at this time), close the PCR lid of the amplification module, then click **"OK"** for amplification detection.

3. Please process according to the following steps for Portable Molecular Workstation (Model: S-Q36A):

3.1 Preparation of consumables and reagents

(1) Take out the Consumables kits and reagents.

(2) Put internal control into the **Well A**, Put Sample Release Reagent into the **Well B**; Put GBS-PCR Mix into the **Well C**; Put GBS-Enzyme Mix into the **Well D**. (The well location information has been marked on the supporting consumables)

(3) Add 20μL sample to be tested or GBS - Positive Control or GBS - Negative Control into the **Well B** (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

3.2 Test Procedure (Refer to user manual of each instrument to adjust the settings.)

3.2.1 Click the "  "and"  " button on the instrument display screen to open the door of the instrument and put the prepared consumables into the designated position of the instrument.

3.2.2 Click the **"New"** on the instrument display screen to enter the new experiment task setting interface.

3.2.3 Select the required experimental project in the drop-down menu of **Lab project**, enter the corresponding task name in the **Task Name** bar, and input and select other items that should be input or selected.

3.2.4 Click **"Submit"** to submit the experimental task and **"OK"** to run the instrument and start the experimental task successively.

4. Please process according to the following steps for Portable Molecular Diagnostic System (S-Q37A/S-Q37B):

4.1 Pre-run preparation

4.1.1 Load the amplification reagent component assembly into the extraction reagent component (Nucleic Acid Extraction-Purification Kit, Reference Number : S50016E-12A) to compose the test reagent cartridge;

4.1.2 Open the seal plug of the sample loading hole, add 350μL sample or GBS-Positive Control or GBS-Negative Control into the sample loading hole (To ensure Diagnostic System have 300μL samples for nucleic acid extraction); or use transfer pipet from the extraction reagent kit to pipette sample into the sample loading hole (When sample enter the lower bubble of transfer pipet indicates enough sample has been taken). Then close the seal plug.

4.2 Test Procedure

4.2.1 Click the **"Specimen"** button on the instrument display screen to open the door of the instrument and enter the new experiment task setting interface.

4.2.2 Put the prepared consumables into the designated position of the instrument.

4.2.3 Enter specimen information, select the required experimental project in the drop-down menu of Experimental project, enter the corresponding task name in the **Task Name** bar, and input and select other items that should be input or selected.

4.2.4 Click **"Submit"** to submit the experimental task and **"OK"** to run the instrument.

Reading test results

1. Result analysis (refer to user manual of each instrument to adjust the settings)

When the reactions are completed, results will be saved automatically. After analysis, adjust Start, End and Threshold values of Baseline (users can adjust them according to the actual situations. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "**Analyze**" to implement the analysis, make sure each parameter satisfies the requirement of the below mentioned "5. Quality Control", then go to "**Plate**" window to record the Ct value and quantitative result.

2. Quality control

The test result is treated as valid if all the conditions in the table below are met for the same test. Otherwise, the test result is treated as invalid and needs to be re-tested.

	GBS-Negative Control	GBS-Positive Control
Ct value	No display; GBS-Internal Control is positive and Ct ≤ 40	Typical S-shape amplification curve and Ct ≤ 38

Reference Range

Through the research on reference values, the Ct reference value for detecting target gene is determined to be 38. The Ct reference value for detecting internal control is 40.

Explanation of Detection Result

Determination of negative and positive result

Conclusion	Ct value of specimen	Ct value of internal control	Amplification curve of specimen
Positive	≤ 38	-	like "S"
Negative	> 38 or N/A	≤ 40	-
Invalid*	> 38 or N/A	> 40 or N/A	-

Note: *This suggests an investigation should be carried out to find out the reasons when Ct value of the internal control is > 40 or N/A and retest it. (If repeated tests still produce invalid results, please contact Sansure Biotech at info@sansure.com.cn)

Limitations of Detection Method

Detection result of specimen is related to specimen collection, processing, delivery and storage quality. Any deviation from the stated procedure may lead to an inaccurate detection result. Cross-contamination during specimen processing may result in a false-positive result.

Product Performance Index

When the kit is used to detect enterprise work references, the conformity rate for both negative and positive reaches 100%. Precision test shows excellent within-lot and between-lot reproducibility with its coefficient of variation of Ct value ≤ 5%. The lower detection limit of this kit is 5.00E+02 copies/mL. It shows no cross-contamination with the other similar pathogens (*streptococcus bovis, streptococcus pneumoniae, streptococcus pyogenes, staphylococcus epidermidis, pseudomonas aeruginosa, escherichia coli, Candida albicans, staphylococcus aureus*, etc.).

Bibliography

1. Ronald S G, Stephanie S, Aline S. Perinatal infections due to group B streptococci[J]. ObstetGynecol, 2004, 104(5): 1062-1076.

2. Jianghong Deng, Yonghong Yang. Research development of molecular biology diagnosis and genotyping of group B streptococci. Chinese Journal of Pediatrics. November 2005, 43(11).

Symbols

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device		Date of Manufacture
	Use-by date		Consult instructions for use
	Temperature Limit		Manufacturer
LOT	Batch Code	REF	Reference Number
Σ	Contains sufficient for <n> tests		Caution
CONTROL −	Negative Control	CONTROL +	Positive Control
ENZYME MIX	Enzyme Mix	INTER CONTROL	Internal Control
VERSION	Version	PCR MIX	PCR Mix
	Do not re-use	21 PAP	PAP21: Not corrugated cardboard
	Keep away from light	PRE	Prepackaging
EC REP	Authorized representative in the European Community	CE	This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices.
UDI	Unique device identifier		

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