Group B Streptococcus Nucleic Acid Detection Kit Manual (Fluorescent PCR Method)

【Product Name】

Generic name: group B Streptococcus （GBS） nucleic acid detection kit (fluorescent PCR method)

【Packaging Specification】

48 tests/box

【Intended Use】

As facultative anaerobic gram-positive streptococcus, GBS normally lodges in the vagina and rectum. It belongs to the conditional pathogenic bacteria and pregnant women can be infected and spread the bacteria to the newborn causing neonatal sepsis, meningitis, pneumonia, etc.

This kit is for the detection of GBS DNA in the samples of reproductive tract and rectal secretions, and can be used for the diagnosis of streptococcus B infection and efficacy monitoring after medication. This test is suitable for 34-37 weeks pregnancy women who have not been given any antibiotics or medicated with suppositories or lotions one week before testing.

【Detection Principle】

This kit uses polymerase chain reaction (PCR) and fluorescence labeled probes to detect specific gene (CAMP) of GBS in clinical samples to determine the presence of GBS. The use of uracil -N-glycosylase and dUTP in the kit is to avoid contamination.

【Kit Contents】

Contents	Specification
10x Concentrated Cleaning Solution	20mL x 1 vial
Extraction Solids	0.1g x 48 vials
GBS-PCR Reaction Solution	1.1mL x 2 vials
Taq DNA Polymerase (5U/μL)	25μL x 1 vial
Uracil N-Glycosylase (UNG) (1U/μL)	10μL x 1 vial
Internal Control	0.5mL x 1 vial
Negative Control	0.5mL x 1 vial
Positive Control	0.5mL x 1 vial

【Storage】

-20±5℃ away from lights, avoid frequent freezing and thawing, valid for 8 months.

【Compatible Instruments】

ABI7500, STRATAGENE Mx3000p etc..

【Packing】

48 tests/kit

【**Sample Collection, Storage and Transport**】

1. Recommended Specimens: reproductive tract secretions and rectal secretions.

2. Recommended Swabs: sterile swabs with head made of polyester, fiber, nylon materials.

3. Specimen Collection: wipe off the excessive secretion in the reproductive tract first, place the sterile swab at the 1/3 lower section of the reproductive tract and gently rotate along the reproductive tract wall to obtain the secretion. Then, carefully insert the same swab into the anus about 2.5cm above the anal sphincter and gently rotate along the wall to obtain the secretion. Put the swab back into the sterile swab sleeve and send to testing spot in a closed condition.

4. Samples should be tested as soon as possible, in case of delay, kept at room temperature for less than 1 day or at 4~8 ℃ for less than 6 days. Ice packs should be used during long distance transportation.

【**Procedure**】

1. Reagent Preparation

1.1 Dilute the 10 x concentrated cleaning solution with sterilized ultra-pure water at 1:9 volume ratio and store it at 4 ℃.

1.2 Briefly spin down the Tag DNA Polymerase and Uracil N - Glycosylase (UNG) and then store them at -20 ℃.

1.3 Calculate the number of PCR reaction tubes N (N = specimen number +2(1 negative control + 1 positive control)), take out the GBS - PCR reaction liquid, put the N x 44.3μL GBS - PCR reaction liquid, N x 0.5μL Taq DNA Polymerase, N x 0.2μL Uracil N - Glvcosylase (UNG) together into one centrifuge tube and vortex, then spin down briefly, and aliquot them into N x PCR reaction tubes with 45μL/tube, tightly cover the tubes with covers and put into 4 ℃ refrigerator.

1.4 Put the extraction solids, positive control, negative control, internal control at the sample operation area with 4 ℃ ambient condition.

2. Sample Processing

2.1 Pipet 1mL cleaning solution into the sample swab tubes, make sure the swabs are complete immerged into the solution, and vortex vigorously for 2 min.

2.2 Sample preparation: Transfer all the liquid into 1.5mL centrifuge tubes and centrifuge at 13000r/min for 5 min, then discard the supernatant, add 1mL cleaning solution and vortex, then centrifuge at 13000r/min for 5 min and discard the supernatant again (This step can be repeated according to the turbidity status of samples). Pipet 50μL cleaning solution and then vortex for 5 min. Add the extracted solids into every sample tube and then vortex vigorously for 5 min.

2.3 Negative control preparation: Pipet 50μL negative control into a 1.5mL centrifuge tube and mix well.

Positive control preparation: pipet 50μL positive control into a 1.5mL centrifuge tube and mix well.

2.4 Spin down the samples briefly, positive control, and negative control, pipet 10μL internal control into every tube then 95 ℃ dry bath for 2 min, ice bath immediately for 2-5 min, and centrifuge for 1 min at 13000r/min, then use the supernatant for PCR amplification.

3. Pipet 5μL of samples into the prepared PCR reaction tubes, and pipet 5μL of positive and negative control into the rest 2 PCR reaction tubes and then spin down briefly after sealing the tubes.

4. PCR Amplification

Put the PCR reaction tubes into the PCR instrument, input the specimen info and then proceed with amplification according to temperature cycle parameters below.

Cycle Parameters: Stage 1: 37° C ---- 2 min Stage 2: 94° C ---- 2 min Stage 3 (10 cycles): 94° C ---- 20 sec 55° C ----45 sec Stage 4 (30 cycles): 94° C ---- 20 sec 55° C ---- 45 sec	GBS detection fluorescein: FAM Internal control fluorescein: Texas Red Reaction Volume : 50μL Fluorescent signal collection: Stage 4

【Reference Value】

Using the instrument matching software to implement automatically analysis, and obtain Ct number (FAM) for all the samples and controls.

1	Specimen (FAM) Ct value ≤ 23 with standard curve	Positive	
2	Specimen (FAM) Ct value = 30, or "No Ct" (Mx3000P) or "Undet" (ABI 7500), Internal Control (Texas Red) Ct value ＜ 30 with nice log amplification curve.	Negative (below LOQ)	
3	Specimen (FAM) 23 ＜Ct value ＜ 30	Vague result area, should be tested two more times	Retest twice, Ct value=30, negative
			Retest twice, at least one Ct value ＜ 30 with standard curve, suspected positive, collect the specimen and test one more time or other detection methods suggested

【Data Analysis and Interpretation】

1. Result analysis condition setting

1.1 ABI 7500 baseline setting: take the fluorescent signal line between cycle 2 and the sample cycle number 3 cycles before threshold is reached as the baseline. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 30 or "Undet"

1.2 STRATAGENE Mx3000P baseline setting: select the fluorescence signal when "Adaptive baseline" is set. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 30 or "No Ct"

2. Quality control standards

Positive and negative control should meet the following standards at the same time, otherwise the test is invalid.

2.1 Negative control, GBS (FAM) Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30 with nice log amplification curve.

2.2 Positive control, GBS (FAM) Ct value ＜23 with nice log amplification curve, internal control (Texas Red) Ct value ≤30.

3. Results Interpretation

3.1 GBS negative (lower than limit of quantitation): FAM Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30 with nice log amplification curve.

3.2 GBS positive: GBS (FAM) Ct value ≤23 with nice log amplification curve, internal control (Texas Red) Ct value ≤30.

3.3 Invalid results, retest is needed. FAM Ct sample = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500)

3.4 For samples with vague results : FAM 23 ＜ Ct sample ＜ 30, retests are suggested.

Note: Test result is for clinical reference only, and should not be taken as the only evidence to diagnose GBS infection. Negative result only means that the DNA content is lower than the LOQ of this kit and should not exclude the possibility of GBS infection. Further confirmation combining other clinical data is highly suggested for making a definite diagnosis when the result is positive.

【Detection Limitation】

This kit is suitable for clinical specimens detection, but the results are affected by the instruments and operation. Therefore, the results are for reference of clinical diagnosis only, and not the only criterion to confirm or exclude disease cases.

【Kit Performance】

The detection lower limit for this kit is 1.0×10^3copies/mL, with a linear range between 1.0×10^8copies/mL and 1.0×10^3copies/mL

It is verified that this kit will not cross-react with other clinical regular pathogens such as candida albicans, colon bacillus, streptococcus pneumoniae, staphylococcus epidermidis etc.

The CV value within same batch and between different batches are both lower than 10%.

The compliance rate is higher than 95% when compared with culture method and sequencing method (gold standard).

【Warnings and Precaution】

Carefully read this instruction before starting the procedure.

1. For in vitro diagnosis use only.
2. Read this manual in detail before the assay, and the assay should be carried out by skilled personnel.
3. Use latex gloves or thin film gloves when handling the PCR tubes.
4. Avoid unnecessary repeated freezing and thawing and keep the PCR solution away from lights.
5. Thoroughly thaw the reagents and spin them down briefly before using.
6. Sterilize centrifuge tubes and pipet tips in high temperature and high pressure before being used.
7. Processing and handling of clinical specimens should be carried out in a biosafety cabinet.
8. After being spin down, the PCR tubes should avoid vortex when being loaded on the PCR instrument.
9. Avoid touching the extraction solids when aspirate DNA temperate.
10. Paraffin is suggested for sealing.
11. Dispose the PCR tubes in sealing airtight plastic bags as biohazard waste after the PCR instrument cool down at room temperature.
12. Dispose the pipet tips into the 10% sodium hypochlorite wasted solution vat and sterilize with other wastes.
13. Sterilize the biohazard safety cabinet by UV lights. After the experiment, clean the biohazard cabinet and pipets with 10% pasteurization, then use 75% ethyl alcohol for cleaning after 10 min.
14. Do not mix-use the reagents from different batches. Use this kit before its expiration.

【References】

《Guidance Principles For In Vitro Diagnosis Reagent Instruction Editing》，SFDA publication

Min Zhu, Jianxia Fan, Linan Cheng. Research progress of perinatal group B streptococcal infection during perinatal stage. February 2005. Chinese Journal of Obstetrics and Gynecology. Volume 40, Issue 2.

Yancey M K. Duff P, Clark P, et al. Peripartum infection associated with vaginal group B streptococcal co lo2nization. Obstetrics & Gynecology, 1994, 84: 816

YanceyM K, Schuchat A ,Brown L K, et al. The Accu2racy of late antenatal screening cultures in predicting genital group B streptococcal colionization at delivery. Obstetrics & Gynecology, 1996, 88: 811.

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