DSPath NGS Multiplex PCR Master Mix

Instruction for Use

【Product Name】

DSPath NGS Multiplex PCR Master Mix

【Cat. No./Spec.】

K030-A (80 rxns); K030-B (400 rxns)

【Product Description】

DSPath NGS Multiplex PCR Master Mix is a multiplex PCR premix for NGS (high throughput sequencing) library preparation, which is applicable for pathogenic microorganisms detection. This product contains various components required for multiplex PCR reactions (except primers and templates). The hotstart high-fidelity DNA polymerase has the characteristics of low mismatch rate and good specificity, and is suitable for Panel multiplicities up to 1000. The optimized buffer system reduces the inhibition of multiple sample interferences on amplification, and is suitable for DNA amplification of various samples such as blood, nose/throat swab, virus culture, etc. DSPath NGS Multiplex PCR Master Mix can quickly complete the amplification library, and is more adapt to products with higher GC content.

【Components】

Component	K030-A (80 rxns)	K030-B (400 rxns)
DSPath NGS Multiplex PCR Master Mix, 2X	1 mL	5 mL

【Storage Condition】

Store at -20°C.

【Application】

NGS library preparation.

【Protocol】

1. Prepare the PCR Reaction Mix

1.1 Allow all reagents to thaw completely. Mix gently by inverting the tube. Spin briefly. Put all reagents on ice.

1.2 The reaction system is formulated according to the following table:

Reagents	Volume	Final Conc.
DSPath NGS Multiplex PCR Master Mix, 2X	12.5 μL	1X
Primer Mix	X μL	0.2 μM each primer [1]
Input DNA	Y μL	0.1~500 ng/reaction [2]
Nuclease-free ddH_2O	To 25 μL	-
Total volume	25 μL	

[1] The recommended range for the final concentration of a single primer is 0.02-0.2 μM. For most reactions, a primer of 0.2 μM gives the desired result.

[2] The recommended amount of Input DNA ranges from 0.1 to 500 ng. If the input is too low, there is the risk of missing detection, and if the input is too high, the amplification efficiency will be reduced.

2. Set the reaction procedure and perform the PCR reaction according to the table below:

2.1 standard procedure

Temperature	Time	Cycle
95°C	3 min	1
95°C	30 sec	35
55°C	90 sec [1]	
72°C	90 sec [2]	
72°C	5 min	1
4°C	hold	1

[1] The annealing time is set according to the number of Panel repetitions, and the reaction time can be increased if the number of Panel repetitions is large.

[2] The extension rate is 1kb/min, and the extension time is set according to the length of the longest fragment, and is not less than 30 sec.

2.1 fast procedure

Temperature	Time	Cycle
95°C	3 min	1
95°C	30 sec	28~35
55°C	30 sec	
72°C	30 sec	
72°C	5 min	1
4°C	hold	1

3. Collect PCR products for subsequent process or store at -20 °C for later use.

This product is for research use only.