VAHTS® AmpSeq Multi-PCR Module V3

NA215

Instruction for Use

Version 21.1

01/Product Description

VAHTS AmpSeq Multi-PCR Module V3 is an amplification reagent based on ultra-multiplex PCR technique to construct amplicon libraries. The initial template for this reagent is 1 - 100 ng. It is compatible with genomic DNA, FFPE samples, cfDNA, etc. This optimized reagent contains enzymes, dNTPs and reaction buffer required during multiplex PCR process, reducing the pipetting operations, making the result more stable. Compared to previous generation (Vazyme #NA205), this reagent has better amplification performance for different input amounts and customized panels. The upgrade ensures the high coverage and high uniformity of the amplicon library. This reagent is compatible with up to 7000 multiplex customized panels. This reagent is suitable for the library preparation for amplicon sequencing, helping researchers and testing personnel to complete ultra-multiplex PCR quickly and easily with high sequencing quality. The reagent has undergone rigorous quality control and functional testing, to ensure the optimal stability and repeatability of library construction.

02/Components

Components	NA215-01 (24 rxns)	NA215-02 (96 rxns)
4 × VAHTS Multi-PCR Mix	120 μl	480 μl

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

This product is suitable for amplicon library preparation with initial DNA template quantities of 1 - 100 ng, and is compatible with various DNA templates:

◇DNA extracted from cells or tissues;

◇DNA extracted from FFPE samples;

◇cfDNA. etc.

For research use only. Not for use in diagnostic procedures.

05/Notes

1. The detection sensitivity of this product is high. To avoid contamination, the first PCR step should be completed in a separate clean area to that used for the subsequent steps.
2. All components of this product should be stored in an environment free of nucleic acids or nuclease contamination, so as to avoid experimental failure.
3. If carrying out the protocol for the first time, we recommend that you set up positive and negative controls at the same time.
4. 4 × VAHTS Multi-PCR Mix is viscous. Prior to use, be sure to invert in order to mix, centrifuge briefly, and pipette these components slowly.

06/ Experiment Process

1. Recommend reaction system:

Components	Volume
DNA template	x μl
Primer Mix*	x μl
4 × VAHTS Multi-PCR Mix	5 μl
Nuclease-free ddH2O	To 20 μl

*The reaction system needs to be adjusted according to the concentration of primers from the customized primer panel. For example, in 20μl system, when the primer panel concentration is 2 ×，the primer mix volume should be 10 μl；when the primer panel concentration is 5 ×，the primer mix volume should be 4 μl；when the primer panel concentration is 10 ×，the primer mix volume should be 2 μl；When designing primers for multiplex PCR, try to keep all primers have the same melting temperature (Tm value). The discrepancies of Tm value for primers could cause poor amplification uniformity. It is recommended that the final concentration of every primer should in the range of 0.02 to 0.2 μM.

▲The 4 × VAHTS Multi-PCR Mix is viscous and needs to be mixed thoroughly and briefly centrifuged. Pipette the solution slowly.

2. Gently pipette up and down to mix (do not vortex), and briefly centrifuge.

3. Recommend reaction procedure:

Temperature	Time	Numbers of cycles
99℃	2 min	
99℃	15 sec	X* cycles
60℃	4 min	
72℃	10 min	
4℃	Hold	

* The number of amplification cycles “x” can be found in the following table:

Primer pairs per pool	General DNA	FFPE/cfDNA
10 - 50	22 - 24	25 - 27
50 – 200	20 - 22	23 - 25
200 - 1,000	17 - 20	20 - 23
≥1,000	15 - 17	18 - 20

Amount of DNA starting material	Adjustment to cycle number
1 ng (300 Copies)	+3
10 ng (3000 Copies)	0
100 ng (30,000 Copies)	-3

▲When the numbers of primer pairs per pool are above 1,000 and 3,000, extend the annealing time to 8 min and 16 min, respectively.

▲If the sample DNA quality is poor, increase the number of cycles appropriately.

07/ FAQ & Troubleshooting

◇Low library yield

①Requantify sample DNA. If the initial template quantity is below the lower limit (1 ng), increase the input template quantity.

②If the template is of poor quality, increase the number of cycles appropriately according to the instructions or use a high quality template instead.

③Please ensure that each step and procedure are carried out in accordance with the instructions, and pay attention to the operation details of each step.

◇High library yield

①Check if the template input quantity is greater than 100 ng. If it is above 100 ng, the template input quantity can be reduced.

②The number of multiplex amplification cycles can be appropriately reduced according to the instructions.

◇Low amplicons uniformity

①It may be caused by degraded sample DNA or inefficient PCR. Use a high-quality template or extend annealing time during the PCR process.

②AT-rich amplicons are under-represented: Double the annealing time, or lower the annealing temperature from 60℃ to 58℃.

③GC-rich amplicons are under-represented: During the first two cycles of the PCR process, increase the annealing temperature from 60℃ to 62℃.

◇Why separate operating areas are required

PCR products are highly susceptible to aerosol contamination, which can lead to inaccurate and unreliable experimental results. Therefore, we recommend that you physically isolate the PCR system preparation area and PCR products purification area, using equipment such as dedicated pipettes, and periodically clean each laboratory area (wipe down with 0.5% sodium hypochlorite or 10% bleach), to ensure the reliability of the experimental results.