VAHTS AmpSeq Multi-PCR Module V3

NA215

Version 22.1

Product Description

VAHTS AmpSeq Multi-PCR Module V3 is an amplification reagent based on super-multiplex PCR technique, used to construct amplicon libraries. It is compatible with gDNA, FFPE DNA, cfDNA, and other sample types with Input DNA of 1 - 100 ng. This optimized reagent contains enzymes, dNTPs and reaction buffer required for multiplex PCR. This reagent has great amplification performance for different input amounts and customized panels, which ensure the high coverage and high uniformity of the amplicon library. It is suitable for the library preparation for amplicon sequencing, helping researchers and technical personnel to perform super-multiplex PCR quickly and easily with. The reagent has undergone rigorous quality control and functional validation to ensure the optimal stability and repeatability of library construction.

Components

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

Storage

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

Applications

This product is suitable for amplicon library preparation with 1 - 100 ng Input DNA, and is compatible with DNA templates derived from different sources:

◇ Cells or tissues;

◇ FFPE samples;

◇ cfDNA, etc.

Notes

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

1. This product has high detection sensitivity. Reagent preparation and template addition should be performed in a separate clean area to avoid contamination.
2. All components of this product should be stored in an environment free of nucleic acids and nuclease contamination.
3. If you are carrying out this protocol for the first time, it is recommended to set up positive and negative controls within the same experiment.
4. 4 × VAHTS Multi-PCR Mix is viscous. Prior to use, please mix it by inversing, centrifuge briefly, and pipette slowly.

Experiment Process

1. Reaction Solution:

Components	Volume	
Template DNA	X μl	
Primer Mix*	X μl	
4 × VAHTS Multi-PCR Mix	5 μl	■
Nuclease-free ddH_2O	To 20 μl	

* If commercial panel primers are used, the reaction solution needs to be adjusted according to the concentration of the primers.When designing primers for multiplex PCR, try to keep the Tm value consistent across all primers to prevent poor uniformity of amplification due to discrepancy in annealing temperature. It is recommended that the final concentration of every primer should be in the range of 0.02 - 0.2 μM.

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

2. Mix the prepared reaction solution thoroughly by gently pipetting instead of vortexing, and centrifuge it briefly to collect the reaction solution to the bottom of the tube.
3. Reaction Program:

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

* For the number of amplification cycles "X", referring to 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

Input DNA	Adjustment of amplification cycles
1 ng (300 Copies)	+3
10 ng (3,000 Copies)	0
100 ng (30,000 Copies)	-3

▲When the numbers of primer pairs per pool are above 1,000 and 3,000, increase the annealing/extension time to 8 min and 16 min, respectively.
▲If the sample DNA quality is poor, increase the number of cycles appropriately.

FAQ & Troubleshooting

◇ Low yield

① Requantify the Input DNA, and confirm whether the amount of Input DNA is accurate. If the amount of Input DNA is below the lower limit (1 ng), please increase it.
② If the template is of poor quality, increase the number of cycles appropriately according to the instructions or use a high-quality template instead.
③ Please ensure that all steps and programs conform to the instructions, and pay attention to the operation details of each step.

◇ High yield

① Check whether the amount of Input DNA is more than 100 ng. If so, reduce the amount of Input DNA.
② The number of multiplex amplification cycles can be appropriately reduced according to the instructions.

◇ Low library uniformity

① It may be caused by degraded sample DNA or inefficient PCR. Use a high-quality template or increase the annealing/extension time of PCR.
② AT-rich amplicons are under-represented: increasing the annealing time, or lower the annealing temperature from 60℃ to 58℃.
③ GC-rich amplicons are under-represented: adding 5% - 10% DMSO in PCR reaction solution.

◇ Aerosol contamination

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 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.