VitaTaq® HS Multiplex Kit is a convenient multiplex formulation of the robust and efficient VitaTaq® DNA Polymerase optimized for applications with several primer pairs in one reaction. The reaction buffer propagates efficient & selective annealing of all primers to the template DNA and protects the enzyme during long PCR experiments and under harsh cycling conditions. The HotStart feature ensures full control over the amplification start and prevents non-specific amplification.

PRODUCT	SIZE	SKU
VitaTaq® HS Multiplex Kit	100 rxn / 50 µl	TRAXSKU1010
	200 rxn/ 50 µl	TRAXSKU1011
	500 rxn / 50 µl	TRAXSKU1012

STORAGE CONDITIONS

Store VitaTaq® HS Multiplex Kit at -20°C, avoid repeated freeze & thaw cycles.

ADDITIONAL MATERIALS REQUIRED

- Nuclease-free PCR tubes or plates and sealing options
- PCR cycler
- Template DNA
- Primers designed for multiplex applications
- Sterile, nuclease-free, DNA-free tubes for preparing the reaction mix
- Nuclease-free dH_2O

REACTION SETUP

1) Thaw VitaTaq® HS Multiplex Kit on ice and mix gently but thoroughly. 5X Multiplex Reaction Buffer already contains an optimal amount of dNTPs.

2) Pre-mix your primer pairs at 5 µM each. In a separate tube, combine equal volumes of all primer pair solutions and mix gently.

   For each primer pair, add 1-2 µl to a 50 µl reaction, e.g. when working with a 10-plex setup, the recommended volume of primers is 10-20 µl for a 50 µl reaction. The exact amount or ratio between primers will be dependent on your individual setup and should be determined individually.

3) Mix the following components in a sterile, nuclease-free tube:

COMPONENT	VOLUME	FINAL CONCENTRATION
5X Multiplex Reaction Buffer	10 µl	1X
Multiplexing Primer Mix	X µl	0.1 µM for each primer
50X Multiplex Enzyme Solution	1 µl	1X
template DNA	1 µl	< 1 µg / 50 µl
nuclease-free dH_2O		to 50 µl

4) Place the reaction in your thermal cycler and immediately start the reaction.

Step	cycles	Temperature	Duration
Hot Start / Initial denaturation	1	95°C	5 min
Amplification	45-50	95°C	10 sec
		57°C	90 sec
Final Extension	1	72°C	5 min
Hold	1	4°C	

5) Analyze the results on an agarose or polyacrylamide gel depending on your product size and required resolution.

General Recommendations & Troubleshooting

- When working with a completely new set of primers, find working conditions for each pair on its own before attempting to multiplex.
- While designing the primers, keep their parameters (T_M, GC-content or length) as similar as possible and check for potential primer oligomerization.
- If amplification is not satisfactory, try
  - increasing the amount of template
  - adding more cycles to your protocol
  - increasing the annealing/extension time
- If band smearing is observed, try increasing the extension / annealing temperature
- The final extension step at 72°C can help the polymerase to finish incomplete amplicons and can increase yield and band sharpness. However, it is optional and can be omitted if causing problems in your application.