AccuStart Long Range SuperMix

Cat. No.	95199-025 95199-100	Size:	25 x 50-µL reactions (1 x 0.313 mL) 100 x 50-µL reactions (1 x 1.25 mL)	Store at -25°C to -15°C

Description

The AccuStart Long Range SuperMix is a 4x, ready-to-use solution that contains all the components for long-range target amplifications, including a blend of two hot-start thermostable DNA polymerases (one with strong proof-reading activity) and an optimized buffer to ensure high efficiency, sensitivity and specificity for amplification of long-range targets. This mix enables routine and easy amplification of up to 24 kb targets from human genomic DNA with high accuracy (> 10-fold better fidelity than Taq) and accommodates targets with broad GC-content (no separate GC buffer needed). Fast cycling conditions (≥2 kb/min) can be implemented for extremely long targets. This product is also capable of amplifying multiple targets simultaneously.

Storage and Handling

Store kit components in a constant temperature freezer at -25°C to -15°C upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Additional reagents and materials that are not supplied

1. Assay primers
2. DNA template
3. Nuclease free water
4. Loading dye (for gel analysis of PCR products)

General Precautions

PCR is a sensitive technique and caution should be taken to avoid potential contaminations either between samples or carryover from prior experiments. Proper sample handling and laboratory techniques are critical:

- Separate pre- and post-PCR areas and use designated area for reaction setup.
- Clean pipettes and work spaces before and after use with either bleach or other decontamination solutions.
- The use of filter plugged pipette tips is highly recommended and care should be taken when pipetting.
- Use PCR-grade nuclease free water and reagents and consumables dedicated for PCR use.

Protocol

Reaction setup

1. Thaw the AccuStart Long Range SuperMix completely and vortex for 3-5 seconds to mix thoroughly. Quick spin to collect contents.
2. Prepare primer mix. A final concentration of 0.2-0.8 µM for each primer is recommended. However, the optimal concentration needs to be empirically determined for each assay, especially for multiplexing reactions.
3. Determine the number of reactions to prepare, including No Template Controls (NTCs). Add 10% extra volume to compensate for the pipetting loss.
4. Follow the table (Table 1) below as a general guidance to set up the reaction mix. It is recommended to make a master mix to minimize variations and potential errors.
5. Close the cap, mix well and spin briefly to bring down reagents.

Table 1: Reaction setup for long-range PCR

Components	Volume/rxn	Final Conc
Nuclease-Free water	to a final 50 µL reaction volume	NA
AccuStart Long Range SuperMix	12.5 µL	1X
Primer mix	X µL	variable
Template	X µL	variable

Thermal Cycling conditions:

Cycling parameters (especially the annealing/extension condition and cycle number) should be empirically determined based on individual assay requirement and DNA input level. Program the cycling conditions based on the recommendations below (Table 2 or 3).

If the annealing temperatures for the primers are above 60°C, a 2-step protocol can be used with annealing/extension at 65°C (Table 2).

Table 2: Cycling conditions (2-step protocol) for long-range PCR

Steps	Temperature	Time	Cycles
Initial activation	95°C	3 min	1
Denaturation	92°C	30 sec	25-35
Annealing/Extension*	65°C	30-60 sec/kb	
Final Extension	72°C	10 min	1
Hold	10°C	Indefinite	1

*If the primer melting temperatures are below 60°C, use the cycling condition below as a guideline (Table 3).

Table 3: Cycling conditions (3-step protocol) for long-range PCR

Steps	Temperature	Time	Cycles
Initial activation	95°C	3 min	1
Denaturation	92°C	30 sec	25-35
Annealing	Approximately 5°C below Tm of primers	30 sec	
Extension	65-72°C*	30-60 sec/kb	
Final Extension	72°C	10 min	1
Hold	10°C	Indefinite	1

*Extension at 68°C works for most targets.

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