UltraMarathonRT

20,000 U/mL | Store at -20°C

TEMPLATE SWITCHING KIT

UltraMarathonRT (uMRT) undergoes an efficient and highly selective form of template switching that can be used to introduce a specific primer binding site to the 5' end of RNAs within a library. Using the Template Switching Oligonucleotides (TSO) as its new template, uMRT then adds a section of known sequence to the 3' end of the resulting cDNA. In this way, cDNA products are tagged at each end, enabling efficient PCR amplification and RNA-seq library preparation.

➔ **To complete the template switching experiment, additional reagents not contained in this kit are necessary.**

Components Not Provided
dNTP mix (10 mM each) • dATP (10 mM) • uMRT dT_{18} & AmpPCR Primer RNaseOUT • KAPA HiFi DNA polymerase (Roche, Cat# KK2102)

uMRT dT_{18}: RT primer:
CCCTCTCTCTCTCTTTCCTCTCTCTTTTTTTTTTTTTTTTT

AmpPCR: PCR primer for cDNA amplification:
CCCTCTCTCTCTCTTTCCTCTCTC

RNAConnect

Distributed by:
CliniSciences Group

Template Switching Quick Start Protocol

ANNEALING RT PRIMERS TO RNA TEMPLATES

1. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Components		Volume (3 µL total)	Final
Primer	uMRT-dT18 (5 µM)	0.5 µL	2.5 pmol
Template RNA	Total RNA	variable	1 ng-20 ng
	poly(A)-RNA	variable	0.1 ng-20 ng
dNTP mix (10 mM each)		0.5 µL	0.5 mM
Nuclease-free Water		added to total 3 µL	

2. Incubate at 95°C for 30 sec and then snap cool on ice to anneal the primer to the template.

REVERSE TRANSCRIPTION

3. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Components	Volume (7 µL total)
2x UltraMarathonRT Buffer	5 µL
UltraMarathonRT Enzyme	0.5 µL
UltraMarathonRT Boost	0.5 µL
RNase Out™ (40 U/µL) (optional)	0.5 µL
Nuclease-free Water	0.5 µL

4. Add RT reaction mix to the annealed RNA and mix gently by tapping the tube (for a total volume of 10 µL).
5. Incubate the mixture at 42°C for 45 min and then hold the tubes at 4°C in a PCR thermocycler.

Above: uMRT Template Switching Kit Components

TEMPLATE SWITCHING

6. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Note: Use a heated lid (55°C) to avoid water evaporation during the reaction.

Components	Volume (10 µL total)
5x Template Switching Buffer	4 µL
UltraMarathonRT Enzyme	2 µL
uMRT-TSO (10 µM)	2 µL
dATP (10 mM)	2 µL

7. Add the template switching reaction mix to the RT reaction tube from step 5 (for a total volume of 20 µL) and gently mix by tapping the tube.
8. Incubate at 42°C for 45 min to catalyze the template switching reaction.
9. Inactivate the enzyme by heating at 95°C for 1 min. then hold the tube at 4°C in a PCR thermocycler.

Note: Use a heated lid (55°C) to avoid water evaporation during the reaction.

Note: The resulting cDNA contains adapters at both ends and can be stored at -20°C until future use.

PRE-AMPLIFY CDNA USING KAPA HIFI

10. Gently mix the following components in a nuclease-free microcentrifuge tube and collect the liquid with a quick spin.

Components	Volume (100 µL total)
Unpurified cDNA product (from Step 9)	20 µL
PCR primer AmpPCR (20 uM)	10 µL
5x GC enhanced buffer	20 µL
dNTP mix (10 mM each)	3 µL
KAPA HiFi DNA Polymerase (1 U/µL)	2 µL
Nuclease-free Water	45 µL

11. Run the below PCR program in a thermocycler.

KAPA HiFi PCR Conditions				
Cycle	Denature	Anneal	Extend	Hold
1	98°C, 2 min	–	–	–
11-15*	98°C, 15 s	62°C, 30 s	72°C, 6 min	–
1	–	–	72°C, 5 min	4°C
∞	–	–	–	4°C

Note: 12 cycles are suggested for 10 ng total RNA or 1 ng mRNA input. 15 cycles are suggested for 1 ng total RNA or 0.1 ng of mRNA.

Note: The resulting pre-amplified cDNA contains adapters at both ends and can be stored at -20°C.

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