TwistAmp® Basic RT *Quick Guide*

Part Number: TABRT01Guide | Revision A

Basic Information

RPA

1) Primers must be 30-35 bases
2) Works best at constant temperature (40-42°C)
3) Amplicons of 80-400bp are preferred

PCR

1) Primers typically 18-25 bases
2) Thermal cycling required
3) Amplicons of 50bp upwards are typical/optimal

Set-up *(single-plex)*[1]

1) Prepare reaction mix in 1.5ml tube:

Primer A (10µM)	2.4 µl
Primer B (10µM)	2.4 µl
Rehydration Buffer	29.5 µl
Template, RNase Inhibitor and dH2O	13.2 µl
(Total Volume	47.5 µl)

Vortex and spin briefly

2) Add reaction mix to freeze-dried reaction. Pipette to mix.
3) Add 2.5 µl of 280mM MgAc (supplied) and mix well to start reaction.

WARNING: RPA REACTIONS START AT ROOM TEMPERATURE AS SOON AS MAGNESIUM IS ADDED.

4) Incubate at 40-42°C for 20-40 minutes.
4b) For low template copy number, remove strip after 5-7 minutes, invert vigorously 8-10 times to mix & spin briefly, replace in heating device.
5) After 20-40 minutes, clean amplicons before running on agarose gels.

WARNING: IF TUBES ARE OPENED AFTER AMPLIFICATION THERE IS A GREAT RISK OF CONTAMINATION OF WORK SURFACES WITH AMPLICON! ENSURE THAT APPROPRIATE AVOIDANCE MEASURES ARE TAKEN!

WARNING: SWITCH OFF HEATED LIDS BEFORE STARTING REACTIONS!

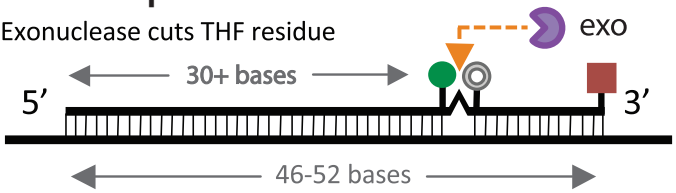
1 See manual for multiplexing

RPA uses TwistDx's proprietary probe systems

RPA does NOT use PCR probe systems

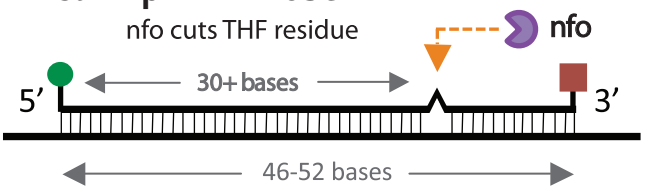
TwistAmp® exo Probe

Exonuclease cuts THF residue



TwistAmp® LF Probe

nfo cuts THF residue



Fluorophore
Quencher
Nuclease
3' block
THF residue

refer to manual for details of probe design

TwistDx

www.twistdx.co.uk