TwistDx™

Unwind DNA's possibilities

TwistAmp® Liquid exo/exo RT

Quick Guide

Part number: LQEXOQG01

Revision 03V4

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Please see instruction and assay design manuals at twistdx. co.uk for information regarding components and storage, assay design, and detailed use.

Instructions are based on 50 µl reaction volumes; if using a different volume, quantities should be adjusted appropriately.

Primer screen set-up (single-plex)[1]

1. Add 2.1 µl of each primer and 0.6 µl of exo probe at 10µM concentration to 0.2 ml PCR tubes.

2. Prepare a pre-master mix (per reaction) in the order below:

2x Reaction Buffer	25 µl
dNTPs[2] + water to (7.2 µl if adding RT[3])[4]	8.2 µl
10x Probe E-mix	5 µl

Vortex and spin briefly.

3. To the pre-master mix, add 2.5 µl 20x Core Reaction Mix[5] (per reaction) to tube lid. Mix by 10x full inversions and spin briefly. If using RNA, add 1 µl 50x RT (per reaction) to tube lid.

4. Add 1 µl 50x Exo (per reaction) to tube lid. Mix by 10x full inversions and spin briefly. Master mix is now complete[6]. Pipette mix before use.

5. Add 41.7 µl[3,4] master mix to primers and probe prepared in tubes (step 1) and pipette mix.

6. Add 2.5 µl of 280mM MgOAc (supplied) and 1 µl template to tube lids[3,4]. DNA/RNA and MgOAc should be kept separate in the tube lid prior to spin-down. Spin in MgOAc/ template and mix well (6x inversions) to start reaction. Spin briefly.

Warning: RPA reactions start as soon as MgOAc is added.

7. Place reactions in a fluorometer and start run: 37-42°C, 20 minutes. For low template copies, remove strip after 4 mins (5 mins for RNA), mix by 6x full inversions and spin briefly, replace in fluorometer.

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Template screen set-up (single-plex)[1]

1. Prepare a primer and probe pre-master mix (per reaction) in the order below:

2x Reaction Buffer	25 µl
dNTPs[2] + water to (7.2 µl if using RT[3])[4]	8.2 µl
10x Probe E-mix	5 µl
Primer A (10µM)	2.1 µl
Primer B (10µM)	2.1 µl
Probe (10µM)	0.6 µl

Vortex and spin briefly.

2. Add 2.5 µl 20x Core Reaction Mix[5] (per reaction) to tube lid. Mix by 10x full inversions and spin briefly.

3. If using RNA, add 1 µl 50x RT (per reaction) to tube lid. Add 1 µl 50x Exo (per reaction) to tube lid. Mix by 10x full inversions and spin briefly. Master mix is now complete[6]. Pipette mix before use.

4. Add 46.5 µl[3,4] master mix to 0.2 ml PCR tubes.

5. Add 2.5 µl of 280mM MgOAc and 1µl template to tube lid[3,4]. DNA/RNA and MgOAc should be kept separate in tube lids prior to spin-down. Spin in MgOAc/template and mix well (6x inversions) to start reaction. Spin briefly.

Warning: RPA reactions start as soon as MgOAc is added.

6. Place reactions in fluorometer and start run: 37-42°C, 20 minutes. For low template copies, remove strip after 4 mins (5 mins for RNA), mix by 6x full inversions and spin briefly, replace in fluorometer.

1 See manual for multiplexing.
2 Suggested final concentration of 1.8mM (total) dNTPs. Optimisation is recommended.
3 If amplifying RNA, users may wish to add RNase Inhibitor together with the template and adjust volumes accordingly.
4 Volumes should be adjusted if adding more/less template and/or MgOAc.
5 Warm to room temperature and pipette mix slowly to ensure homogeneity.
6 Master mix may appear cloudy, this is normal.

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