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## TwistAmp™ Liquid exo

## Quick Guide

Part number: INLQEXO

Revision 3



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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

  dNTPs² + water³ to

  8.2 µl

  10x Probe E-mix

  5 µl

  Vortex and spin briefly.
- To the pre-master mix, add 2.5 µl 20x Core Reaction Mix<sup>4</sup> (per reaction) to tube lid. Mix by 10x full inversions and spin briefly.

- Add 1 µl 50x exo (per reaction) to tube lid. Mix by 10x full inversions and spin briefly. Master mix is now complete<sup>5</sup>. Pipette mix before use.
- Add 41.7 μl<sup>3</sup> 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<sup>3</sup>. DNA 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, mix by 6x full inversions and spin briefly, replace in fluorometer.

### Template screen set-up (single-plex)1

 Prepare a primer and probe premaster mix (per reaction) in the order below:

 2x Reaction Buffer
 25 μl

 dNTPs² + water³ to
 8.2 μl

 10x Probe E-mix
 5 μl

 Primer A (10μΜ)
 2.1 μl

 Primer B (10μΜ)
 2.1 μl

 Probe (10μΜ)
 0.6 μl

 Vortex and spin briefly.

- Add 2.5 µl 20x Core Reaction Mix<sup>4</sup> (per reaction) to tube lid. Mix by 10x full inversions and spin briefly.
- 3. Add 1 µl 50x Exo (per reaction) to tube lid. Mix by 10x full inversions and spin briefly. Master mix is now complete<sup>5</sup>. Pipette mix before use.
- 4. Add 46.5 μl<sup>3</sup> master mix to 0.2 ml PCR tubes.
- Add 2.5 µl of 280mM MgOAc and 1µl template to tube lid3. DNA 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.

6. Place reactions in fluorometer and start run: 37-42°C, 20 minutes. For low template copies, remove strip after 4 mins, 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 Volumes should be adjusted if adding more/ less template and/or MgOAc.
- 4 Warm to room temperature and pipette mix slowly to ensure homogeneity.
- 5 Master mix may appear cloudy, this is normal.

