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# TwistAmp™ Liquid Basic

## Quick Guide

Part number: INLQBAS

Revision 4



### TwistAmp™ Liquid Basic Quick Guide

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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 2.4 µl of each primer 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

  9.2 µl

  10x Basic E-mix

  5 µl

  Vortex and spin briefly.
- 3. 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. Master mix is now complete<sup>5</sup>. Pipette mix before use.

- Add 41.7 μI<sup>3</sup> of master mix to primers prepared in tubes (step 1) and pipette mix.
- 5. Add 2.5 µl of 280mM MgOAc (supplied) and 1 µl template to tube lids³. DNA and MgOAc should be kept separate in the tube lid prior to spindown. 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. Incubate at 37-42°C for 20-40 minutes. For low template copies, remove strip after 4 mins, mix by 6x full inversions and spin briefly, replace in heating device.
- 7. After step 6, clean amplicons before running on an agarose gel.

Warning: Opening tubes post amplification will risk contamination of work surfaces with amplicon. Ensure appropriate control measures are taken.

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

- 1. Prepare a primer pre-master mix (per reaction) in the following order: 2x Reaction Buffer 25 µl dNTPs² + water³ to 9.2 µl 10x Basic E-mix 5 µl Primer A (10µM) 2.4 µl Primer B (10µM) 2.4 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. Master mix is now complete<sup>5</sup>. Pipette mix before use.
- 3. Add 46.5 µl<sup>3</sup> master mix to 0.2 ml PCR tubes
- 4. Add 2.5 µl of 280mM MgOAc and 1 µl template to tube lid³. DNA and MgOAc should be kept separate in the tube lid prior to spin-down. Spin in MgOAc/template, mix well (6x inversions) to start reaction. Spin briefly.

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

- Incubate at 37-42°C for 20-40 minutes. For low template copies, remove strip after 4 mins, mix by 6x full inversions and spin briefly, replace in heating device.
- **6.** After step 5, clean amplicons before running on an agarose gel.

Warning: Opening tubes post amplification will risk contamination of work surfaces with amplicon. Ensure appropriate control measures are taken.

- 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.

