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## TwistAmp<sup>™</sup> Liquid Basic

# Quick Guide

Part number: INLQBAS Revision 4



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### TwistAmp<sup>™</sup> Liquid Basic Quick Guide

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)<sup>1</sup>

- 1. Add 2.4 µl of each primer at 10µM concentration to 0.2 ml PCR tubes.
- Prepare a pre-master mix (per reaction) in the order below:
  2x Reaction Buffer 25 µl
  dNTPs<sup>2</sup> + water<sup>3</sup> to 9.2 µl
  10x Basic 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. Master mix is now complete<sup>5</sup>. Pipette mix before use.

- Add 41.7 µl<sup>3</sup> of master mix to primers prepared in tubes (step 1) and pipette mix.
- 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 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)<sup>1</sup>

- Prepare a primer pre-master mix (per reaction) in the following order: 2x Reaction Buffer 25 µl dNTPs<sup>2</sup> + water<sup>3</sup> 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.
- Add 46.5 μl<sup>3</sup> master mix to 0.2 ml PCR tubes.
- Add 2.5 µl of 280mM MgOAc and 1 µl template to tube lid<sup>3</sup>. 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.

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- 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.

TwistDX	PMS 185 C	Part Number: INLQBAS	
Liquid Basic QG	Red	Revision 4	
Size: 8.268 in x 5.827 in	Black		
		Date of Revision:	
	PMS 7541 C	4.2 2020/06/26	
	Gray 1 - 10%		