

## TwistAmp® nfo Kit

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### Quick Guide

Part number: TANFO02Guide

Revision E

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Please see **instruction** and **assay design manuals** at [twistdx.co.uk](http://twistdx.co.uk) for information regarding kit components and storage, assay design, detailed use and multiplexing.

## Set-up (single-plex)

1. Prepare reaction mix in 1.5ml tube:

Primer A (10 $\mu$ M)	2.1 $\mu$ l
Primer B (10 $\mu$ M)	2.1 $\mu$ l
TwistAmp® nfo probe (10 $\mu$ M)	0.6 $\mu$ l
Primer Free Rehydration buffer	29.5 $\mu$ l
Template and water to	13.2 $\mu$ l
(Total volume	47.5 $\mu$ l)

Vortex and spin briefly.

2. Add reaction mix to a TwistAmp® nfo reaction. Pipette to mix.

3. Add 2.5  $\mu$ l of 280mM Magnesium Acetate (MgOAc) (supplied) and mix well to start reaction.

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

4. Incubate at 40 °C for 20 minutes. For low template copy number, remove strip after 4 minutes, vortex and spin briefly, replace in heating device.

5. After 20 minutes, for analysis by lateral flow, dilute reaction products as specified by the lateral flow consumable of choice (see **instruction manual**).

**Note:** 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.

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TwistAmp® exo probes can also be used with this kit (see [instruction manual](#)).

## Kit Positive Control Set-up (single-plex)

1. Prepare reaction mix in 1.5ml tube:

Positive control primer/probe mix	8 µl
Primer Free Rehydration buffer	29.5 µl
Positive control DNA template	1 µl
Water	9 µl
(Total volume	47.5 µl)

Vortex and spin briefly.

2. Add reaction mix to a TwistAmp® nfo reaction. Pipette to mix.

3. Add 2.5 µl of 280mM Magnesium Acetate (MgOAc) (supplied) and mix well to start reaction.

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

4. Incubate at 39°C for 20 minutes. For low template copy number, remove strip after 4 minutes, vortex and spin briefly, replace in heating device.

5. After 20 minutes, for analysis by lateral flow, dilute reaction product 1/50 with PBST and load 10 µl onto the sample pad of Milenia HybriDetect 1 strip.

6. Place strip in PBST running buffer.

**Note:** 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.

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Definitions. As used in this section, “kit” means the items described in this manual (the “Manual”) and supplied by TwistDx to a purchaser (the “Recipient”). “Materials” means all biological and chemical materials supplied as part of the kit. “Information” means all written information supplied as part of the kit, information relating to the kit made available through TwistDx’s website, and any verbal or written information concerning the kit or its use provided by any employee or agent of TwistDx.

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