TwistFlow® Salmonella *Quick Guide*

Part Number: TFSAL02Guide | Revision A

RPA

- Includes primers & probe to amplify & detect the INVA gene of *Salmonella enterica* as well as probe & template for detection of an internal control.
- Optimal constant temperature for reactions is 40°C.
- Kit includes 2-analyte detection Milenia lateral flow strip.
- Sample preparation method included. Optional method without sample preparation.

Set-up with integrated lysis

1) Culture or elute *Salmonella enterica* in recommended medium[1].
2) Prepare a reaction mix for each reaction in 1.5ml tubes: Lysis Buffer (LyB) 20µl
   Sample culture/eluate
   (+ dH2O if applicable) 5µl
3) Mix and leave to lyse for 10 seconds.
4) Add 25ul of Neutralisation buffer (NB) and mix. The reaction mix is ready for measurement now.
5) Add reaction mix to freeze dried reaction. Pipette to mix[2]. Spin briefly.
6) Place reaction strip in pre-equilibrated heating device at 40°C for 10 minutes. After 4 minutes remove reaction strip, invert vigorously 8-10 times to mix, spin briefly & return to heating device.
7) For analysis by lateral flow, dilute reaction products 1/50 with PBST and load 10µl onto a provided strip.
8) Place strip in 200µl PBST running buffer and leave for 1-2 minutes.
9) Salmonella has been detected if the Biotin line is positive (see score matrix).

WARNING: 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!

WARNING: TWISTFLOW® SALMONELLA KITS ARE FOR R&D PURPOSES ONLY!

WARNING: SWITCH OFF HEATED LIDS BEFORE STARTING REACTIONS!

1 *Recommended enrichment media compatible with direct lysis and fluorescence detection are BPW (Oxoid CM0509) and Nutrient broth (Oxoid CM0001). For elution of Salmonella for later cultivation (swabs, contaminated specimen etc.) we recommend Maximum recovery diluent (Oxoid CM0733). If no further cultivation is required elution can be carried out in water or directly with lysis buffer and water in a mixing ratio of 20(LyB) + 5(H2O) as outlined under section 2. Be aware that higher elution volumes decrease the bacterial titer per volume.*

2 *As Mg(OAc)2 is included in Neutralisation buffer the RPA reaction is instantly initiated once it comes into contact with the reaction pellet, which greatly increases the risk of cross-contamination. It is possible to add all 50µl of reaction mix to the strip cap and spin and vortex to resuspend the pellets.*

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- Optimal constant temperature for reactions is 40°C.
- Kit includes 2-analyte detection Milenia lateral flow strip.
- Sample preparation method included. Optional method without sample preparation.

Standard set-up (without lysis)

1) Culture *Salmonella*, lyse & purify DNA (elute DNA into dH2O).
2) Prepare a reaction mix for each reaction in 1.5ml tubes:

Rehydration buffer (PIRB)	37.5µl
Sample DNA + dH2O	10µl [1]
(Total Volume	47.5 µl)

3) Add reaction mix to freeze dried reaction. Pipette to mix.
4) Add 2.5 µl Mg(OAc)2 (450mM) to reaction strip caps and firmly place on tubes [2].
5) Vigorously invert tubes 8-10 times to mix. Spin briefly.
6) Place reaction strip in pre-equilibrated heating device at 40°C, 10 minutes total. After 4 minutes remove reaction strip, invert vigorously 8-10 times to mix, spin briefly and replace in heating device.
7) For analysis by lateral flow, dilute reaction products 1/50 with PBST and load 10µl onto the sample pad of strip.
8) Place strip in 200µl PBST running buffer and leave for 1-2 minutes.
9) Salmonella has been detected if the Biotin line is positive (see score matrix).

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1 *Or 1µl positive control + 9µl dH2O*

2 *Mg(OAc)2 can be included in the reaction mix, but this greatly increases the risk of cross-contamination as the RPA reaction is instantly initiated by the Mg. It is possible to add all 50µl of reaction mix and Mg to the strip caps and spin and vortex to resuspend the pellets.*

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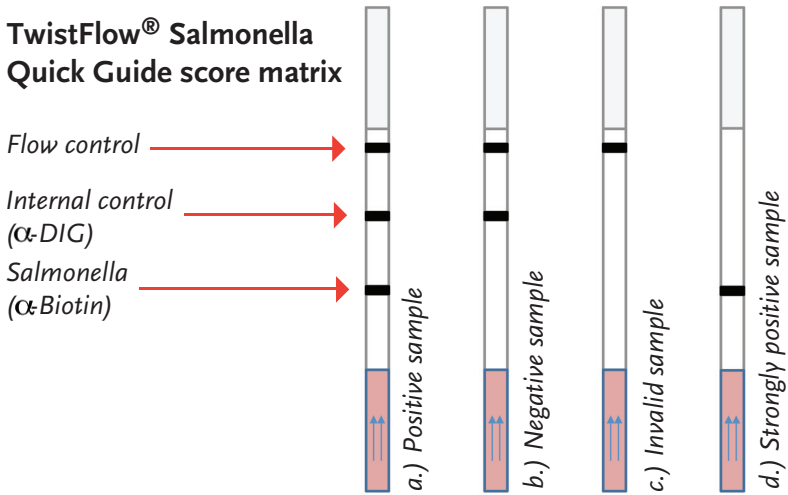
The TwistFlow® Salmonella kit uses a TwistAmp® nfo probe

TwistFlow® Salmonella Quick Guide score matrix



To find out how to design your own TwistAmp® assays, please visit ***www.twistdx.co.uk***

TwistDx

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