

TwistGlow® Salmonella Quick Guide

Part Number: TGSALo2Guide | Revision A

RPA

- Includes primers & probe to amplify & detect the INVA gene of *Salmonella enterica* (FAM) as well as primers, probe and template of an internal control (SIMA)(HEX), detection in TAMRA channel).
- Optimal constant temperature for reactions is 40°C.
- Sample preparation method included. Optional method without sample preparation.

Set-up with integrated lysis

- 1) Culture or elute *Salmonella enterica* in recommended medium¹.
- 2) Prepare a reaction mix for each reaction in a 1.5ml tube:
Lysis Buffer (LyB) 20 µl
Sample culture/eluate(+ dH₂O if applicable) 5 µl
- 3) Mix and leave to lyse for 10 seconds.
- 4) Add 25µl of Neutralisation Buffer (NB) and mix. The reaction mix is now ready for measurement.
- 5) Add reaction mix to freeze dried reaction. Pipette to mix². Spin briefly.
- 6) Place strip in Twista^{®3} and start run: 40°C, 10 minutes.
- 7) Remove strip after 4 minutes, invert vigorously 8-10 times to mix. Spin briefly, replace in Twista[®].

WARNING: RPA REACTIONS START AT ROOM TEMPERATURE AS SOON AS MAGNESIUM IS ADDED.

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: TWISTGLOW® 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 titre per volume.

2 As Mg(OAc)₂ is included in the Neutralisation buffer the RPA reaction is instantly initiated once the reaction mix contacts the reaction pellet. This greatly increases the risk of cross-contamination. Tips should be ejected into a 10% bleach solution. It is possible to add all 50µl of reaction mix to the strip caps and spin and vortex to resuspend the pellets.

3 Other heated fluorometers (eg plate readers or real-time PCR machines) may also be used. The TwistGlow® Salmonella probe is FAM and the internal control probe SIMA (HEX) labelled (detection in TAMRA channel), so any alternative device should be set up with this in mind.

TwistGlow® Salmonella Quick Guide

Part Number: TGSALo3Guide | Revision A

RPA

- Includes primers & probe to amplify & detect the INVA gene of *Salmonella enterica* (FAM) as well as primers, probe and template of an internal control (SIMA(HEX), detection in TAMRA channel).
- Optimal constant temperature for reactions is 40°C.
- 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 a 1.5ml tube:

Rehydration buffer (PIRB)	37.5µl
Sample DNA + dH ₂ O	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 strip caps and firmly place on tubes².
- 5) Vigorously invert tubes 8-10 times to mix. Spin briefly.
- 6) Place strip in Twistax^{®3} and start run: 40°C, 10 minutes.
- 7) Remove strip after 4 minutes, invert vigorously 8-10 times to mix. Spin briefly, replace in Twistax[®].

WARNING: RPA REACTIONS START AT ROOM TEMPERATURE AS SOON AS MAGNESIUM IS ADDED.

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

WARNING: SWITCH OFF HEATED LIDS BEFORE STARTING REACTIONS!

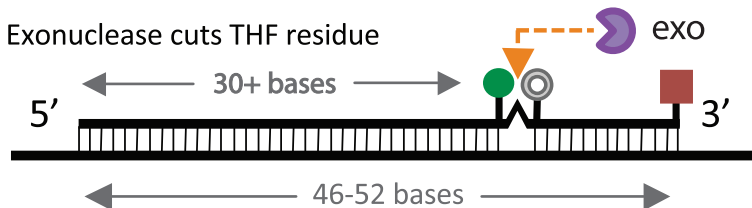
1 Or 1µl positive control + 9µl dH₂O.

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.

3 Other heated fluorometers (eg plate readers or real-time PCR machines) may also be used. The TwistGlow® Salmonella probe is FAM and the internal control probe SIMA (HEX) labelled (detection in TAMRA channel), so any alternative device should be set up with this in mind.

TwistGlow[®] Salmonella Quick Guide

The TwistGlow[®] Salmonella kit uses a TwistAmp[®] exo probe



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|--|---|--|
|  Fluorophore |  Quencher |  3' block |
|  Exonuclease |  THF residue | |

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

TwistDx

www.twistdx.co.uk