

# TRANSIA® PLATE *Salmonella* Gold

SA0180

NF VALIDATION Certification TRA 02/08 - 03/01  
AOAC Performance Tested Methods<sup>SM</sup> 010602

## Intended Use

TRANSIA PLATE *Salmonella* Gold is an ELISA kit intended to be used by food microbiologists for the detection of *Salmonella* genus. The assay reliably recovers and detects low levels of *Salmonella* in a variety of foods and feeds.

TRANSIA PLATE *Salmonella* Gold has been certified by AFNOR for food products, feed products, pet food products and industrial production environmental samples (except breeding samples).

TRANSIA PLATE *Salmonella* Gold has been validated by the AOAC Research Institute under the Performance Tested Methods<sup>SM</sup> Program (PTM 010602) for the following tested matrices: raw ground turkey, Brie cheese, cooked chicken, raw milk, cantaloupe, sausages, raw shrimps, yogurt, mayonnaise, shell eggs, frozen red berries and currants, bean sprouts, raw ground beef, smoked trout, fresh pasta, milk chocolate, ground black pepper, cake mix, dry milk-based infant formula and dry food for pets.

TRANSIA PLATE *Salmonella* Gold has a method modification (PTM 010602) for a next-day assay in the following tested matrices: ready-to-eat poultry, roast beef, chicken carcass rinsate, raw spinach, raw pasta, raw almonds, stainless steel, sealed concrete and plastic.

## Assay Principle

There are three enrichment options for this kit:

1. Enrichment procedure comprises two stages, buffered peptone water (BPW) as a pre-enrichment medium followed by Rappaport-Vassiliadis Soya (RVS) as a selective enrichment medium. (AFNOR (02/08-03/01), AOAC PTM 010602)
2. Enrichment procedure comprises mEHEC as enrichment medium (processed foods and environmental surfaces, AOAC PTM 010602)
3. Enrichment procedure comprises mEHEC + novobiocin as a pre-enrichment medium followed by trypticase soy broth (TSB) as a selective enrichment medium (for unprocessed foods, AOAC PTM 010602)

## Materials

### Kit components

- Microtiter plate with divisible strips, 96 wells (8 wells x 12 strips) – 1 pc
- Lid for the microtiter plate – 1 pc
- Negative control – ready to use – 1 x 10 mL
- Positive control – ready to use – 1 x 8 mL
- Washing buffer – concentrated 20X – 1 x 60 mL
- Conjugate: anti-*Salmonella* antibodies conjugated to peroxidase – ready to use – 1 x 16 mL
- Substrate: TMB – ready to use – 1 x 57 mL
- Stop solution: H<sub>2</sub>SO<sub>4</sub> – ready to use – 1 x 57 mL
- Extraction reagent – ready to use – 1 x 12.5 mL

## Equipment required but not provided

For sample and reagent preparation:

- Scales and weighing vessels
- Homogenizer according to ISO 7218 (e.g., stomacher)
- Stomacher bags, preferably with a filter or Erlenmeyer flasks (500 mL)
- Tubes (20 mL) for the subcultures
- Sterile tubes (50 mL), polypropylene, for Novobiocin solution (optional)
- Magnetic stirrer
- Air incubator at  $37 \pm 1$  °C
- Air incubator at  $41.5 \pm 1$  °C or preferably a water bath with circulating water at  $41.5 \pm 0.5$  °C
- Vortex mixer
- Test tubes (5 or 10 mL) resistant to 100 °C
- Graduated cylinder 1 L
- Water bath at 95 – 100 °C (boiling water)

For the immunoenzymatic test:

- Micropipette: 100 – 1000 µL
- Wash bottle or preferably an automatic microplate washer
- Absorbent paper
- Multipipette with 2.5 and 5 mL combitips or a multichannel micropipette: 100-200 µL
- Microtiter plate reader – single or double beam reading 450 nm filter and reference filter  $\geq 595$  nm

For confirmation of samples:

- Air incubator at  $37 \pm 1$  °C

## Material required but not provided

For sample preparation

- Distilled water
- Buffered peptone water (BPW)
- Rappaport-Vassiliadis Soya (RVS) broth

**Note:** Because of a high magnesium chloride content, Oxoid CM669 is not recommended in the Nordic countries.

- Novobiocin sodium salt (optional)
- UHT skimmed milk (environmental samples only)

For sample preparation for AOAC Performance Tested Method (010602)

- mEHEC media
- Trypticase soy broth (TSB)
- Novobiocin sodium salt

For confirmation of samples:

- Selective agar plates such as Xylose Lysine Deoxycholate (XLD), ASAP™
- Biochemical identification gallery

## Storage Conditions

The kit components should be stored at 2 – 8 °C. The kit expiry date is shown on the box label.

## Safety

Good laboratory practice (refer to EN ISO 7218 standard) should be employed when using this kit. Safety clothing should be worn and skin contact with reagents avoided. Do not ingest.

Material and safety data sheets (SDS) are available on request.

Contaminated material should be disposed according to local, state and federal regulations.

## Test Procedure

### Preparation of Reagents

**Important:** Allow the reagents to come to room temperature (15 – 30 °C). Remove them from the box at least one hour before use.

Have all reagents and samples ready for use so that the various materials can be added to the wells without delay.

Shake each vial manually or mix with a vortex mixer before use.

*Do not interchange reagents between kits with different batch numbers.*

### Dilution of washing buffer 20X

The dilution can be done in advance or during the first incubation step. See Assay Procedure, Step 3.

1. Dilute the washing buffer 20X in distilled water 20-fold by combining 60 mL washing buffer 20X and 1140 mL of distilled water. Mix well.
2. Fill the washing device.
3. Store the washing buffer 1X at room temperature (15 – 30 °C) for 1 month or at 2 – 8 °C for a maximum of three months.

## Sample Preparation

### AFNOR or PTM

1. Homogenize X grams or X mL of the sample with 9X mL of Buffered Peptone Water (BPW, Appendix A) in a stomacher bag or Erlenmeyer flask, following the special requirements of EN ISO 6579-1:2017 standard. In the context of NF Validation, test portions weighing more than 25 g have not been tested.
2. Incubate at  $37 \pm 1$  °C for 16 – 20 h.
3. Homogenize and inoculate 0.1 mL of the pre-culture broth in 10 mL of Rappaport-Vassiliadis Soya (RVS, Appendix A).

Optional Novobiocin Procedure (RVS+n):

Inoculate 0.1 mL of the pre-culture broth in 10 mL RVS+n (Appendix A). For environmental samples only, add 0.1 mL of UHT skimmed milk to 10 mL of RVS+n prior to subculture.

4. Incubate for 18 – 24 h at  $41.5 \pm 1$  °C in an air incubator, or preferably at  $41.5 \pm 0.5$  °C in a water bath with circulating water.

PROCEED TO SAMPLE INACTIVATION

## Sample Preparation

### AOAC® PERFORMANCE TESTED METHODS

#### Enrichment for environmental surfaces and processed food types (RTE meat and RTE poultry)

For ready-to-eat meat and poultry, add 25 g test portion or 325 g test portion to 225 mL or 1.3 L respectively prewarmed (42 °C) mEHEC (Appendix A). Stomach/masticate for 2 minutes and incubate for 22- 30 h at 41.5 – 42.5 °C for 25 g samples and 24-32 h at 41.5 – 42.5 °C for 325 g samples.

For environmental surfaces, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swabs by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of prewarmed mEHEC, respectively. Mix well and incubate 20 – 28 h at 41.5 – 42.5 °C.

#### Pre-enrichment for raw and unprocessed foods (select foods) and poultry carcass rinsate

For poultry rinsate, add 30 mL poultry rinsate to 30 mL of prewarmed (42 °C) mEHEC with Novobiocin (mEHEC+n, Appendix A). Mix well and incubate 20 – 28 h at 41.5 – 42.5 °C.

For raw vegetables, nuts, and fresh pasta add 25 g or 375 g test portion to 225 mL or 1.5 L respectively of prewarmed (42 °C) mEHEC+n. Stomach / masticate for 2 minutes and incubate 20 – 28 h at 41.5 – 42.5 °C for 25 g samples and 24 – 32 h at 41.5 – 42.5 °C for 375 g samples.

#### Selective Enrichment for raw and unprocessed foods and poultry rinsate

Transfer 0.1 mL pre-enriched broth to 10 mL of prewarmed Tryptic Soy Broth with Novobiocin (TSB+n, Appendix A).

Vortex to mix well. Incubate TSB+n for 6 – 24 h at 41.5 – 42.5 °C (preferably in a water bath).

PROCEED TO SAMPLE INACTIVATION

## Sample Inactivation

Mix final sample. Combine 0.1 mL of Extraction Reagent and 1 mL of the final enrichment broth in a clean test tube and vortex. Heat enrichment broth with extraction reagent in a water bath at 95 –100 °C (boiling water) for 15 – 20 min, then cool to room temperature. Retain remaining enrichment broth for confirmation of a positive or doubtful result, if necessary.

#### Storage of samples

If the test cannot be performed immediately after the final enrichment medium incubation time (8 – 24 h), incubated broth can be stored up to 48 hours at 2 – 8 °C before heat inactivation and ELISA test. The category “ready-to-eat” and “ready-to-reheat” products are excluded for cold storage for RVS+n.

Do not store the incubated final enrichment medium more than 2 days at 2 – 8 °C before performing the confirmation of positive or doubtful results.

## Performance Characteristics

#### Specificity

No false-positive results were found in the AFNOR Certification.

#### Special application

This kit has been validated in-house for Gemini automated systems. The results obtained are equivalent to the results obtained using the manual procedure.

For further instructions on how to use the kit in the automatic system, see the relevant User’s Manual.

## Appendices

Worksheet – Immunoassay results (P/N 56000 / Lit 20516011)

## Result Interpretation

### Test validation

- The optical density of the positive control, OD (PC) must be equal to or higher than 0.700.
- The optical density of the negative control, OD (NC) must be equal to or lower than 0.150 (for double beam reading) or 0.200 (for single beam reading).
- If the controls do not meet these requirements, the results are invalid.

### Positive threshold

Calculate the positive threshold as the average of the negative controls plus 0.20:

$$\text{Positive threshold} = \frac{\text{OD}(\text{NC1}) + \text{OD}(\text{NC2})}{2} + 0.20$$

### Negative threshold

Calculate the negative threshold as the positive threshold multiplied by 0.9.

### Negative result

The sample is considered negative for *Salmonella* if its optical density is lower than the negative threshold.

### Doubtful result

The sample is considered as doubtful if its optical density is lower than the positive threshold but equal to or higher than the negative threshold.

This result should be confirmed by streaking onto selective media plates followed by a biochemical identification (see Confirmation of positive results).

### Positive result

The sample is considered positive for *Salmonella* if its optical density is equal to or higher than the positive threshold.

## Confirmation of Positive Results

### Isolation of *Salmonella*:

In the context of NF Validation, isolate all samples identified as positive by TRANSIA PLATE *Salmonella* Gold. From the retained, incubated RVS or RVS+n broth (before Sample Inactivation), streak for isolation onto appropriate selective agar plates according to EN ISO 6579-1:2017/Amd 1:2020.

### Confirmation of *Salmonella* colonies:

Isolated *Salmonella* must be confirmed. Choose one of the following:

- A. In the context of NF Validation, confirm colonies by either of the following methods:
  1. Performing biochemical confirmations, according to ISO 6579-1:2017/Amd 1:2020 (including purification of typical colonies).
  2. Using PCR nucleic probes, as described in ISO 7218:2024 standard, from isolated colonies (including purification of typical colonies, if needed).
- B. In the context of ISO general rules, confirm colonies by:

Using any other ISO 16140-6:2019 certified method (e.g., MALDI-TOF), the principle of which is different from TRANSIA PLATE *Salmonella* Gold. The confirmation protocol of the second validated method shall be followed entirely. All steps preceding the step from which the confirmation is performed shall be common to both methods (Transia and second validated method).

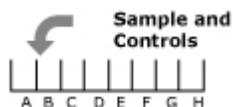
In the event of discordant results (presumptive positive with the alternative method, non-confirmed by one of the means described above), the laboratory must follow the necessary steps to ensure validity of the result obtained.

## Assay Procedure

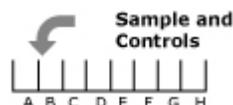
Have all the reagents and samples ready for use and at room temperature so that materials can be added to the wells without delay. Shake the reagent vials before use. The washing step is very important. When washing, direct a strong stream at the bottom of the well. Wipe the microtiter plate lid clean before each use.



1. Attach the required number of strips to the plate: two wells for the negative control, one for the positive control and one well for each sample. Return the unused strips to the resealable bag containing dehydrating agent and close it tightly. Write the position of the samples on the work sheet.



2. Distribute **100 µL** of the controls and the samples into the assigned wells. Vortex samples prior to transferring 100 µL to wells. Cover the plate with the lid.



3. Incubate at room temperature (15 – 30 °C) for 1 h ± 10 min. Prepare the washing buffer – see Preparation of Reagents.



4. Holding the plate firmly, shake out the contents of the plate by briskly flicking your wrist. Rinse each well, keep the washing buffer in the wells for one minute (for the first two washings). Empty the plate over a container and then remove the remaining liquid by inverting the plate onto a paper towel and tapping the plate firmly several times. Repeat the washing for a total of **five times**.



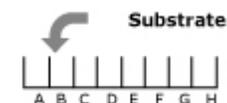
5. Add **100 µL** of the conjugate to each well. Be careful not to touch the wells with the tip. Cover the plate with the lid.



6. Incubate at room temperature (15 – 30 °C) for 30 ± 5 min.



7. Holding the plate firmly, shake out the contents of the plate by briskly flicking your wrist. Rinse each well, keep the washing buffer in the wells for one minute (for the first two washings). Empty the plate over a container and then remove the remaining liquid by inverting the plate onto a paper towel and tapping the plate firmly several times. Repeat the washing for a total of **five times**.



8. Add **100 µL** of the TMB substrate to each well using a multipipette and cover the plate.



9. Incubate at room temperature (15 – 30 °C) for 15 ± 2 min.

**Stop Solution**



10. Add **100 µL** of the stop solution to each well, following the same order used when the substrate was added. Mix the contents of the wells thoroughly to ensure complete color conversion. The blue turns to yellow.

11. Read the optical densities at 450 nm using a plate reader (blank on air), use a reference filter ≥ 595 nm for double beam reading.

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## Appendix A – Enrichment Media Recipes

### **Buffered Peptone Water (BPW)**

Follow the manufacturer's instructions for preparation of media.

### **Tryptic Soy Broth (TSB)**

Follow the manufacturer's instructions for preparation of media.

### **Modified EHEC (mEHEC®) Broth**

Pre-warm sterile deionized water at 42 °C overnight. On day of use, aseptically transfer 31.6 g of mEHEC® media into 1 L of pre-warmed sterile water. Gently mix to rehydrate the media. Use prepared broth within 6 h. Alternatively, mEHEC® media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to rehydrate the media, dispense into desired volume and autoclave at 121 °C for 15 min. Broth must be pre-warmed to 42 °C prior to sample addition.

### **Rappaport-Vassiliadis Soya (RVS) Broth**

Follow the manufacturer's instructions for preparation of media.

### **mEHEC® with Novobiocin (mEHEC®+n)**

Prepare mEHEC® as described above. On day of use, add 0.9 mL of 0.45% Novobiocin solution to 225 mL mEHEC® (6 mL of 0.45% Novobiocin solution to 1.5 L mEHEC). For poultry carcass rinsate, add 120 µL of 0.45% Novobiocin solution to 30 mL mEHEC.

### **TSB with Novobiocin (TSB+n)**

Prepare TSB as described above. On day of use, add 90 µL of 0.45% Novobiocin solution to 10 mL TSB.

### **RVS with Novobiocin (RVS+n)**

Prepare RVS as described above. On day of use, add 25 µL of 0.45% Novobiocin solution to 10 mL RVS. For environmental samples only, add 0.1 mL of UHT skimmed milk to 10 mL of RVS+n prior to subculture.

### **0.45% Novobiocin Solution**

Dissolve 0.225 g of novobiocin sodium salt in 50 mL of sterile deionized water. Store in dark at 2 – 8 °C.

**NF Validation certificate granted by AFNOR Certification for TRANSIA® PLATE Salmonella Gold in all food products, feed products, pet food products and industrial production environmental samples (except breeding samples) in relation to the reference method described in the ISO EN 6579-1/A1 international standard in accordance with EN ISO 16140-2 (2016). For more information about the end of validity of the NF VALIDATION certification, please refer to the certificate TRA 02/08-03/01 available on the website <http://nf-validation.afnor.org/en>.**



**TRA 02/08-03/01**

**ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS**

<http://nf-validation.afnor.org/en>

## Manufacturing Entity

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