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 both AFNOR Certification and NordVal for foods, feeds and environmental samples.

TRANSIA PLATE Salmonella Gold has been validated by the AOAC Research Institute under the Performance Tested Methods 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 cat.

ASSAY PRINCIPLE
The 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.

MATERIALS
Kit components
- Microtiter plate with divisible strips, 96 wells (8 wells x 12 strips) – 10 pc
- Lid for the microtiter plate – 1 pc
- Negative control – ready to use – 2 x 13 mL
- Positive control – ready to use – 2 x 8 mL
- Washing buffer – concentrated 20X – 1 x 800 mL
- Conjugate: anti-Salmonella antibodies conjugated to peroxidas – ready to use – 2 x 57 mL
- Substrate: TMB – ready to use – 2 x 57 mL
- Stop solution: H₂SO₄ – ready to use – 2 x 57 mL
- Extraction reagent – ready to use – 2 x 60 mL

Equipment required but not provided
For sample and reagent preparation:
- Scales and weighing vessels
- Homogenizer according to ISO 7218 (i.e. 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 ± 1°C
- Air incubator at 41.5 ± 1 °C or preferably a water bath with circulating water at 41.5 ± 0.5 °C
- Vortex
- 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
- Microtiter plate reader – single or double beam reading 450 nm filter and reference filter ≥ 595 nm

For confirmation of samples:
- Air incubator at 37 ± 1 °C

Material required, but not provided
For sample preparation
- Distilled water
- Buffered peptone water (BPW): available from BioControl
- Rappaport-Vassiliadis Soya (RVS): available from BioControl
Note: Because of a high magnesium chloride content, Oxoid CM0669 is not recommended in the Nordic countries.

- Novobiocin sodium salt (optional): available from BioControl
- UHT skimmed milk (environmental samples only)

For confirmation of samples:
- Selective agar plates such as xylose lysine deoxycholate, 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) 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 are available on request.

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

ASSAY MODIFICATION SINCE LAST RENEWAL
An optional subculture in RVS with novobiocin was added to the enrichment procedure.

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 with a Vortex before use.

Do not interchange reagents between kits with different batch numbers.

Preparation of the BPW, RVS enrichment broth and plating media
Follow the manufacturer’s instructions.

Preparation of the Novobiocin solution (optional, not applicable to PTM 010602)
0.45% Novobiocin solution (optional): Dissolve 0.225 g Novobiocin sodium salt in 50 mL sterile distilled water. Store away from light at 2 – 8 °C.

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 50 mL washing buffer 20X and 950 mL of distilled water.
2. Mix and fill the washing device.
3. Store the washing buffer 1X at room temperature (15 – 30 °C) for a month or at 2 – 8 °C for a maximum of three months.

Preparation of samples
For preparations of sample suspensions, follow instructions of EN ISO 6887 standards.

1. Homogenize X grams or X mL of the sample with 9X mL of buffered peptone water in a stomacher bag or Erlenmeyer flask, following the special requirements of EN ISO 6579:2002 standard. In the context of NF Validation, test portions weighing more than 25 g have not been tested.
2. Incubate at 37 ± 1 °C for 16 – 20 h.
3. Homogenize and inoculate 0.1 mL of the pre-culture broth in 10 mL of RVS.

Optional Novobiocin Procedure (not applicable to AOAC PTM 010602): Dispense 25 µL of 0.45% Novobiocin solution in 10 mL of RVS to make RVS+n. For environmental samples only, add 0.1 mL of UHT skimmed milk to 10 mL of RVS+n prior to subculture. Inoculate 0.1 mL of the pre-culture broth in RVS+n tube.

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

5. Vortex RVS (or RVS + n) tubes and combine 0.1 mL of Extraction Reagent and 1 mL of the RVS (or RVS + n) 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 RVS (or RVS + n) tubes for confirmation of a positive or doubtful result is necessary.

Storage of samples
- If the test cannot be performed immediately after the RVS (or RVS + n) broth incubation time (8 – 24 h), incubated broth can be stored up to 48 hours at 2 – 8 °C before heat inactivation and ELISA test (this does not apply to the AOAC RI validation as the additional storage was not included in the study). The category “ready-to-eat” and “ready-to-reheat” products are excluded for cold storage for RVS + n.
- Do not store the incubated RVS or RVS + n medium more than 2 days at 2 – 8 °C before performing the confirmation of positive or doubtful results.
Sample preparation flow charts

X g or X mL of sample to 9X mL Buffered Peptone Water (1st step enrichment)

Homogenize and Incubate: 16 – 20 h at 37 ± 1 °C

0.1 mL of culture into 10 mL RVS or RVS+n (2nd step enrichment)

Incubate: 18 – 24 h at 41.5 ± 1 °C

1 mL of culture + 0.1 mL of extraction reagent per each tube

Heat shock: 15 – 20 min at 95 – 100 °C (in boiling water)

TRANSIA® PLATE Salmonella Gold

Performance characteristics

Specifity

No false positive samples were found in the AFNOR Certification or NordVal validation studies.

Special application

This kit has been validated in-house for TRANSIA Elisamatic (Gemini, ThunderBolt) and TRANSIA 4U, and the results obtained are equivalent to the results obtained using the manual procedure. The protocols are available on request. For further instructions on how to use the kit in the automatic system, see the relevant User’s Manual.

An alternate enrichment protocol (TAG 24 Salmonella method, BioControl P/N AK0180) has been certified by AFNOR Certification for the detection of Salmonella spp. in 24 h with TRANSIA PLATE Salmonella Gold (NF Validation Certificate TRA 02/9 - 07/07). This certification does not include the use of the Extraction Reagent. See the specific Package Insert of TRANSIA ADDITIVE Salmonella Gold 24 h for further information.

Validations


AOAC-RI: Producer supplied samples of this test kit model were independently evaluated by the AOAC Research Institute and were found to perform to the producer’s specifications as stated in the test kit’s descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the Performance Tested Methods SM. Certificate number 010602. Reference method EN ISO 6579:2002.

The AOAC-RI study showed the high levels of inclusivity (96.1%) and exclusivity (100%) of the assay. The overall method agreement calculated on confirmed results between TRANSIA® PLATE Salmonella Gold and the ISO 6579:2002 methods was 98.8%.

With a sensitivity rate of 99.3% and a specificity rate of 97.4% the TRANSIA PLATE Salmonella Gold method appeared to give comparable results to the reference method for the 20 tested matrices (for details on the 20 matrices, see Intended use, page 1).

Notice:
The reader used to interpret the results of the TRANSIA PLATE Salmonella Gold assay should be routinely calibrated and maintained as specified by the equipment manufacturer and in accordance with the laboratory’s quality program.

Appendices

Worksheet – Immunoassay results P/N 56000

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

TPSG (BioControl P/N SA0190) Protocol

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

Positive threshold = \[\frac{\text{OD (NC1)} + \text{OD (NC2)}}{2} + 0.20\]
TAG24 Additive (AK0180) Protocol

If running the TAG24 additive protocol, calculate the positive threshold as the average of the negative controls plus 0.11:

\[ \text{Positive threshold} = \frac{\text{OD (NC1)} + \text{OD (NC2)}}{2} + 0.11 \]

**Negative threshold**

TPSG and TAG24 Protocol

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

**Negative samples**

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

**Doubtful samples**

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

**Positive samples**

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

**Confirmation of positive results**

In the context of NF Validation, all samples identified as positive by TRANSIA PLATE *Salmonella* Gold must be confirmed by one of the following ways:

1) Using the non-heated RVS or RVS+n broth, streak on selective agar plates, followed by biochemical identifications according to EN ISO 6579 (including the purification step).

2) Using nucleic probes as described in EN ISO 7218 standard from isolated colonies (including or not the purification step).

3) Using any other NF Validation certified method, the principle of which is different from TRANSIA PLATE Salmonella Gold. The detection protocol of the second validated method shall be followed entirely. All steps that are before the step from which the confirmation is done shall be common to both methods.

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 the various 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 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 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.

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.

For more information about the NF VALIDATION certification’s expiration date, please refer to the certificate TRA 02/08 - 03/01 available at http://nf-validation.afnor.org/en or contact BioControl systems.

Product Warranty

Biocontrol Systems, Inc. (BCS) warrants this product to be free from defects in materials and workmanship, when stored under labeled conditions and used as intended until the expiration date stated on the package. BCS agrees during the applicable warranty period to replace all defective products after return to BCS. BCS shall not have obligation under this Limited Warranty to make replacements which result, in whole or in part, from negligence of the Buyer, or from improper use of the products, or use of the product in a manner for which it was not indicated. Buyer shall notify BCS of any products which it believes to be defective during the warranty period. At BCS option, such products shall be returned to BCS, transportation and insurance prepaid. BCS shall replace any such product found to be defective, at no charge. Should BCS examination not disclose any defect covered by the foregoing warranty, BCS shall so advise Buyers and dispose of the product in accordance with Buyer’s instruction.