For laboratory use only

BioKits

F.A.S.T. IMMUNOSTICK MEAT SPECIES SCREENING KIT

For the qualitative detection of species content in raw, processed and mixed meats

Store at 2–8°C

Contents

1. F.A.S.T. (FOOD ANALYTE SCREENING TESTS) are available for the following species:

Red: Cow (Neogen item: 502135B)

Orange: Horse (Neogen item: 502136Y)

Yellow: Pig (Neogen item: 502137W)

Green: Sheep/Goat (Neogen item: 502138U)

Blue: Poultry (Neogen item: 502139S)

Rabbit* (Neogen item: 502140J)

Kangaroo* (Neogen item: 502141G)

Turkey* (Neogen item: 502186H)

Product numbers listed above refer to five-test packs for the relevant species. F.A.S.T. kits consist of five of these packs (i.e., 25 tests) plus an Accessory Pack containing a product insert, worksheet, ABTS Concentrate, Stop Solution and 25 disposable Pasteur pipettes. Single or mixed species kits can be ordered in any combination of species packs.

**Special manufacture – please inquire.*

2. KIT CONTENTS (STORE AT 2–8°C)

A. **F.A.S.T. IMMUNOSTICKS:** Colour-coded tubes each containing a white plastic paddle coated with species-specific Capture Reagent in a stabilising and preservative buffer solution. Tube caps are colour coded according to test type (see above). **Store upright.**

B. **ENZYME REAGENT:** Colour-coded tubes containing species-specific Enzyme Reagent.

C. **COLOUR DEVELOPMENT REAGENT** (CDR) tubes (no colour code).

D. **ACCESSORY PACK**

- ABTS concentrate: One 5 mL dropper bottle containing concentrated Colour Reagent (aqueous ABTS).
- Stop solution: One 10 mL dropper bottle containing aqueous sodium fluoride.
- 25 disposable plastic pipettes
- Kit insert

2.1 MATERIALS REQUIRED BUT NOT PROVIDED

A. Clean (preferably disposable) glass or plastic containers or Stomacher bags for sample extraction.

B. Cold tap water (or alternatively deionised or distilled water) is required for extraction and washing stages.

C. Scale (Neogen item 9427)

D. 250 mL graduated cylinder (Neogen item 9368)

E. Timer (Neogen item 9426)

F. Wash bottle (Neogen item 9366) (optional)

G. Spectrophotometer with 414 nm filter (optional)

H. F.A.S.T. Immunostick racks (Neogen item 201167Q) hold up to 40 tubes during testing (optional).

3. NOTES ON THE METHOD

STORAGE: All reagents except the Colour Development Reagent tubes and ABTS Concentrate are supplied ready-for-use as outlined in the test method. F.A.S.T. species test kits and F.A.S.T. Accessory Packs should be stored refrigerated when not being used but brought to room temperature (19–21°C) before use.

DROPPER BOTTLES: Remove cap and add dropwise additions by carefully inverting and squeezing gently so that a drop slowly forms at the end of the nozzle and falls into the appropriate tube. A single drop is required for ABTS concentrate; four drops for Stop Solution.

SAMPLE/REAGENT ADDITIONS: Dropwise additions are outlined here for simplicity. If preferred, micropipettes can be used for additions as follows:

- Sample extract–200 µL
- Positive Control solutions–750 µL
- ABTS Concentrate–40 µL
- Stop Solution–200 µL.

The use of such pipettes will improve both accuracy and precision of test results.

MIXING: Test performance is improved if tube contents are mixed during standing times. Mixing can be achieved by screwing and unscrewing the tube cap 3–4 times. **Do not invert tubes** at any stage since this may cause reagents to become trapped in the screw cap where they may be difficult to remove during normal washing.

TIMINGS: Accurate timing provides more reproducible test results and reduces day-to-day and operator variation. **10 minute** standing times will be sufficient in most circumstances; however, if the test is being performed below normal room temperature (19–21°C) it may be necessary to use longer (**20 minute**) incubation times.

WATER QUALITY: Cold tap water is recommended for use during the sample extraction and washing stages of the procedure. However, water quality can vary and may have an impact on test performance. In areas where there is a high level of chlorination or high levels of minerals (hard water), Neogen recommends the use of deionised or distilled water during washing and sample extraction stages as an alternative to cold tap water.

WASHING: Ensure all white plastic surfaces (i.e., the faces of the paddle, paddle stem and white surface of the F.A.S.T. immunostick lid) are washed thoroughly. Washing times have been optimised and should not be shortened but longer washing times will not affect results.

COLOUR REAGENT: Colour Development Reagent should be prepared (just prior to commencing the first additions) by adding one drop of ABTS Concentrate to each Colour Development Reagent tube used in that run. Re-cap the tubes.

COLOUR STABILITY: After addition of Stop Solution, enzyme activity slows. If the optical density is to be determined by a spectrophotometer at 414 nm, this should be carried out within **20 minutes** from addition of the stop solution.

4. F.A.S.T. TEST METHOD

Sample preparation:

Homogeneity of the sample will improve test results. Frozen swarf samples, previously minced, blended or other similar materials need no further preparation; intact meats should be minced or finely chopped to improve the extraction process.

1. **Meat samples**: Add a suitably representative sample to approximately 10 times its weight of water and mix well for **5–6 minutes**. Alternatively, the sample can be chopped and stomached with water in a strong plastic bag for **5–6 minutes**. Fatty meat samples may require longer extraction timings. Please refer to **Recommendations** section on page 6.
2. Allow extract to settle for **2–3 minutes**. A layer of liquid will appear above the sample (this layer of liquid is referred to as the sample supernatant) or a thin slurry will be obtained that may or may not settle out. This liquid/slurry is the sample extract.

5. TEST PROCEDURE

1. Select the required number and species combination of F.A.S.T. Immunosticks, Enzyme Reagent and CDR tubes.
2. Mark the Immunostick caps or tubes to ensure adequate sample identification.
3. Prepare CDR by adding one drop of ABTS Concentrate to the required number of CDR tubes. Replace cap.
4. Unscrew the cap of the first F.A.S.T. Immunostick tube and, using a clean disposable pipette, add 5–6 drops of sample supernatant to the liquid in the tube. Replace paddle and mix contents by rotating cap 3–4 times. Tighten cap. Allow the first tube to stand for **10 minutes**.

   NOTE: If the same extract is to be tested for other species, repeat additions to other (different colour-code) F.A.S.T. Immunostick tubes at set intervals (**1 minute**). Discard pipette. Add other sample extracts to F.A.S.T. Immunostick tubes at minute intervals.
5. Remove first paddle and wash for **10 seconds**. Ensure all white surfaces of the paddle, paddle stem and the white surface of the immunostick lid are washed thoroughly. Please refer to washing and water quality sections on page 3 for further information. Shake to remove excess water. Screw washed paddle into an appropriate (same colour code) Enzyme Reagent tube. Rotate cap to mix.
6. Allow paddles to stand in Enzyme Reagent tubes for **10 minutes**.
7. Repeat at minute intervals for all tubes.
8. Remove first paddle and wash for **30 seconds**. Ensure all white surfaces of the paddle, paddle stem and the white surface of the immunostick lid are washed thoroughly. Please refer to washing and water quality sections on page 3 for further information. Shake to remove excess water. Remove cap from a prepared CDR tube (no colour code) screw in washed paddle and rotate cap 3–4 times to mix.
9. Allow paddles to stand in CDR for **10 minutes**.
10. Repeat at minute intervals for all tubes.

11. Add four drops of Stop Solution to all tubes at minute intervals. Mix well to stop colour development and evenly distribute colour.
12. Observe the tubes for a noticeable colour change. A negative result is indicated by a clear or very light green colour. A positive result is indicated by a darker green colour.
13. **OPTIONAL:** If necessary, colour development can be determined by measuring the absorbance of the solution using a spectrophotometer.
14. Set spectrophotometer to zero at 414 nm using water as a blank.
15. Using a clean cuvette, measure and record the absorbance of one CDR tube – see step 3.
16. Measure the absorbance of the unknown samples and record on the worksheet provided. Those having an absorbance of more than 3–5 times that of the Colour Development Reagent should be regarded as likely to contain at least 1% of the meat of interest. Alternatively, a 1% extract of the target meat can be used as a positive control and the absorbance of unknown meat samples can be compared against the optical density of the 1% extract.

6. POSITIVE CONTROL (NEOGEN ITEM 502304E)

The use of the positive control is optional but may help to ensure proper quality control of the method (each control sufficient for two tests).

- Positive Control (for Cow/Horse/Pig/Sheep/Poultry) – Neogen item 502304E

At the sample addition stage of the **Test Procedure** (step 4), use the Positive Control in place of an unknown sample extract as follows:

1. Remove screw cap/paddle of the relevant F.A.S.T. Immunostick and **discard** the liquid contents.
2. Add 15–20 drops (750 µL) of control to the tube and replace paddle.
3. Mix contents by rotating cap 3–4 times. Tighten cap and incubate for **10 minutes**. Proceed with remainder of test from step 5 in the **Test Procedure.**

7. RECOMMENDATIONS

1. Ensure the laboratory and/or defined working area(s) and all equipment are thoroughly cleaned to reduce the possibility of cross-contamination from one sample to another or one method to another.
2. Ensure all equipment is maintained/checked and calibrated as appropriate at defined intervals.
3. F.A.S.T. test kits are designed for qualitative screening of raw meat and raw meat products. The F.A.S.T. test is designed to detect species-specific serum albumin proteins. These proteins degrade upon heating, therefore F.A.S.T. test kits are not suitable for screening of cooked products for species identification. (For screening of cooked products for species identification, please contact a Neogen representative for further details on our range of speciation products.) Curing and brining processes may denature serum albumin proteins. Neogen recommends that validation work is carried out on products that have undergone these processes before using the F.A.S.T. tests.
4. It is recommended that cold tap water, or alternatively deionised or distilled water, is used during sample extraction. However, saline solution (0.9% (0.15 M) sodium chloride, preferably in purified water) also can be used as an alternative extraction solution at the same ratio of 1:10, weight of meat sample to saline solution.
5. Cold tap water is recommended for use in sample extraction and washing stages of the test procedure. However, water quality can vary and may impact on test performance. In areas where there is a high level of chlorination or high levels of minerals (hard water), Neogen recommends the use of deionised or distilled water during washing and sample extraction stages as an alternative to cold tap water.
6. Extraction of fatty samples may take longer and extraction time should be increased to **10 minutes** or until the meat sample is homogenous throughout the aqueous extraction solution. A fat layer or crust may form above the sample supernatant and care should be taken when pipetting to obtain the sample extract. In these circumstances, Neogen recommends pipetting just below the fatty layer to obtain the sample supernatant. Alternatively, the fatty layer or crust may be removed prior to pipetting the sample supernatant.
7. In addition to raw meat, other body tissues may give a positive response in the appropriate species assay. This should be taken into consideration when testing products that contain tissues from more than one species. For example, beef fat and beef sausage casings may all give a positive response in the cow F.A.S.T. and egg may give a positive response in the poultry F.A.S.T. assay.
8. The F.A.S.T. tests are designed to be run at room temperature (19–21°C). Running at a higher laboratory temperature may result in increased colour development and, therefore, a higher background colouration in negative samples.

9. The F.A.S.T. tests are designed to use 10 minute incubations during the test procedure. Longer incubation times may result in greater colour development and may result in higher background colouration in negative samples.

10. The F.A.S.T. tests are optimised so that one drop of ABTS concentrate should be added to the CDR tube. If no ABTS is added, there will be no colour development in any of the tests. If more than one drop of ABTS is added to each tube, the colour development will be stronger and this will result in increased colour development in negative samples.

11. F.A.S.T. tests are qualitative tests and are designed for screening purposes only. It is recommended that positive samples are tested using a confirmatory method.

12. Ensure spectrophotometers, if used, are set to the correct wavelength (414 nm) and regularly calibrated.

13. To assess the impact of laboratory and water conditions on the F.A.S.T. tests, it is recommended to run a blank water sample alongside routine samples for colour development comparison.

14. In the unlikely event that problems persist and/or you need further assistance with troubleshooting, contact Neogen Europe Ltd. or your local distributor and, if possible, provide the following information:

    - Kit type and batch number.
    - Brief details of the samples being tested and preparation/extraction methods.
    - Immunoassay equipment used; laboratory temperature.
    - Copies of raw data, including data reduction output if appropriate.

This ensures that Neogen Europe Ltd. can respond promptly and fully to your needs.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's test kits, on Neogen's website at www.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

TERMS AND CONDITIONS

For Neogen's full terms and conditions, please visit www.neogen.com/Corporate/termsconditions.html.

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TESTING KITS AVAILABLE FROM NEOGEN

Natural toxins
- Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria
- *E. coli* 0157:H7, *Salmonella*, *Listeria*, *Listeria monocytogenes, Campylobacter, Staphylococcus aureus*

Sanitation
- ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens
- Almonds, crustaceans, eggs, gliadin, hazelnut, lupine, milk, mustard, peanuts, sesame, soy, walnuts

Genetic modification
- CP4 (Roundup Ready®)

Ruminant by-products
- Meat and bone meal, feed

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