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, milk and plasma

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: 502135 B)

Orange: Horse (Neogen item: 502136 Y)

Yellow: Pig (Neogen item: 502137 W)

Green: Sheep (Neogen item: 502138 U)

Blue: Poultry (Neogen item: 502139 S)

Lilac: Rabbit* (Neogen item: 502140 J)

Grey: Kangaroo* (Neogen item: 502141 G)

Brown: Turkey* (Neogen item: 502186 H)

White: Goat* (Neogen item: 502184 M)

Product numbers listed above refer to 5-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).

Also available:

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

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 BUFFER** tubes (no colour code).

D. **ABTS CONCENTRATE:** One 5 mL dropper bottle containing concentrated Colour Reagent (aqueous ABTS).

E. **STOP SOLUTION:** One 10 mL dropper bottle containing aqueous sodium fluoride.

F. **REQUIRED BUT NOT PROVIDED**: Clean (preferably disposable) glass or plastic containers or stomacher bags for sample extraction.

3. NOTES ON THE METHOD

STORAGE: All reagents except the Colour Development Buffer tubes and ABTS Concentrate are supplied ready-for-use as outlined in the test method. They should be stored refrigerated when not being used but brought to room temperature 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 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 timings, while not vital, give more reproducible test results and reduce 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.

WASHING: Wash using cold, running tap water. Ensure all white plastic surfaces, particularly the faces of the paddle, are washed well. Washing times have been optimised and should not be shortened but longer washing times will not affect results.

COLOUR REAGENT: Colour Development Reagent (CDR) should be prepared (just prior to commencing the first additions) by adding one drop of ABTS Concentrate to each Colour Development Buffer tube used in that run. Re-cap the tubes; if the spectrophotometric protocol is to be used (see steps 16–18 in the **Test Procedure** section) read one of the CDR tubes at 414 nm (ABS).

COLOUR STABILITY: After addition of Stop Solution, enzyme activity virtually ceases and colour is stable for 2–3 days. If colour measurement is required but not possible on-site, tubes must be stored tightly capped.

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. Milk needs no pre-dilution and can be added direct to the capture reagent tube.

1. Add a suitably representative sample to approximately 10 times its weight of water and mix well. Alternatively the sample can be chopped and stomached with water in a strong plastic bag.
2. Allow extract to settle for **2–3 minutes**. A layer of liquid will appear above the sample or thin slurry will be obtained which 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 Immunosticks, Enzyme Reagent and Buffer tubes.
2. Mark Immunostick caps to ensure adequate sample identification.
3. Prepare Colour Development Reagent (CDR) by adding one drop of ABTS Concentrate to the required number of CDR tubes; replace cap.
4. Unscrew the cap of the first Immunostick tube and, using a clean disposable pipette, add 5–6 drops of sample extract to the liquid in the tube. Replace paddle and mix contents by rotating cap 3–4 times; tighten cap.
5. If the same extract is to be tested for other species, repeat additions to other (different colour-code) Immunostick tubes as in step 4 above at set intervals (**1 minute**). Discard pipette.
6. Add other sample extracts to Immunostick tubes at minute intervals.
7. Allow first tube to stand for **10 minutes**.
8. Remove first paddle and wash for **10 seconds**; shake to remove excess water. Screw washed paddle into an appropriate (same colour code) Enzyme Reagent tube. Rotate cap to mix.
9. Repeat at minute intervals for all tubes.
10. Allow paddles to stand in Enzyme Reagent tubes for **10 minutes**.
11. Remove first paddle and wash for **30 seconds**; shake to remove excess water. Remove cap from a prepared CDR tube (no colour code) screw in washed paddle and rotate cap to mix.
12. Repeat at minute intervals for all tubes.
13. Allow paddles to stand in CDR for **10 minutes**.
14. **OPTIONAL:** Add four drops of Stop Solution to all tubes at minute intervals. Mix well to stop colour development and evenly distribute colour.
15. Observe the tubes for noticeable colour change (from clear/very pale green to darker green solution) which indicates a positive result. Record results (as "+" or "-") on the worksheet provided.
16. **OPTIONAL:** If necessary, colour development can be quantitated by measuring the absorbance of the solution using a spectrophotometer.
17. Set spectrophotometer to zero at 414 nm using water as a blank.
18. Using a clean cuvette, measure and record the absorbance of one CDR tube – see step 3 above. This is most conveniently done during the incubation with Enzyme Reagent, step 10 above (replace Colour Development Reagent in its tube so that it can be used later).
19. 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.

Positive Controls for use with F.A.S.T. raw meat test kits:

- Positive Control (for Cow/Horse/Pig/Sheep/Chicken) – Neogen item 502403 E
- Rabbit – Neogen item 502049 T
- Goat – Neogen item 502190 S
- Kangaroo – Neogen item 502127 Z
- Turkey – Neogen item 502188 D

The use of these reagents helps ensure proper quality control of the method (each control sufficient for two tests).

Homologous species controls (e.g., Positive Control in Cow/Horse/Pig/Sheep/Chicken / Poultry F.A.S.T. tests, Rabbit Control in the rabbit test etc.) will give a positive response (green colour) indicating that both operator technique and reagent performance are acceptable.

The testing of **heterologous** controls (e.g., Positive Control in rabbit, goat, etc. tests, Rabbit Control in any other F.A.S.T. test, etc.) should always yield a negative result (little or no change in colour; absorbance less than three times that of the CDR) indicating that the washing procedure has been sufficiently thorough.

6. USE OF THE CONTROLS

At the sample addition stage of the Test Method (sections 4–6 above) 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. Proceed with remainder of test as described above.

7. TROUBLESHOOTING

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. Plate shakers (if used) should be set at 700–800 rpm. Ensure that, at this setting, well contents are agitated but do not approach the rim of the well. If shaker speed is too slow, OD450 levels may be low. If too high, imprecision may occur.
4. Ensure plate readers/spectrophotometers, if used, are set to the correct wavelength and regularly calibrated. 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 NeogenEurope Ltd. can respond promptly and fully to your needs.

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