For laboratory use only

BioKits

Raw Species Identification Test Kit

Sandwich enzyme immunoassay for the qualitative detection of species content in uncooked meat and meat products

Information for:

Four species (cow/pig/sheep/poultry) 96-well assay (Neogen item 902025D)

Single species (horse) 48-well assay (Neogen item 902122M)

Store at 2–8°C

Contents

1. INTRODUCTION

The identification of meat species is performed for various reasons, both economic and ethnic, to prevent the substitution of meat with unsuitable or inferior species, or in religious communities where particular meat is proscribed.

A variety of methods, both biochemical and immunological, have been applied to the problem, including electrophoresis, fatty acid patterns, gel diffusion and nephelometry. Enzyme immunoassay (EIA) techniques have also been implemented in the analysis of food and EIA's based on serum proteins have been developed for meat speciation. The BioKits range of raw species identification test kits employ the principles of EIA and are sensitive and specific tests designed to aid in the resolution of species content in uncooked meats and meat products.

2. PRINCIPLE

The BioKits Raw Species Identification Test Kit is a direct, sandwich type enzyme immunoassay consisting of two main procedures:

Sample preparation and extraction: Samples are minced and then extracted using saline solution; followed by dilution prior to the enzyme immunoassay procedure.

Enzyme immunoassay: A direct, sandwich enzyme-linked immunosorbent assay (ELISA) where colour development is proportional to the amount of albumin in the extract and a qualitative estimate of meat species type may be made by visual inspection or using a spectrophotometer or plate reader.

3. SPECIFICATIONS

Limit of detection:	<1% cow, pig*, sheep, poultry (Neogen item 902025D) <1% horse (Neogen item 902122M) LOD was determined as the lowest concentration of homologous serum albumin consistently detected by the test. *The pig limit of detection can be lowered (<0.2%) by following the high sensitivity protocol (available upon request)
Number of determinations:	Four species (Neogen item 902025D): 96 wells total, 24 wells (including controls) per species Horse assay (Neogen item 902122M): 48 wells total
Sample preparation:	Mincing, stomaching and dilution
Time required:	Sample preparation/extraction time approximately: **60 minutes** (5 samples) Enzyme immunoassay incubation time: **30 minutes**
Specificity:	Species specific albumins

Cross-reactivity: These tests have been checked against a wide variety of other meats/sera; some related species cross-react with the beef, poultry and sheep tests. Offal samples (lung, heart, liver, kidney, fat) behave in a very similar manner to lean meat. Serum/plasma is detected at a dilution of 1/1,000,000 or below in diluent. Negative results were obtained when testing foods such as wheat gluten and flour, cornflour, peanut and soya. Casein and whey powder (1% solutions) react positively in the cow test. Bovine albumin is detected at a level of 50 ng/mL or below. Milk adulteration (cow, sheep) can be detected down to a level equivalent to 1% or less. Chicken egg yolk is strongly positive in the poultry test, whereas egg white is positive at a significantly lower level. A validation report is available with detailed cross-reactivity information.

4. KIT COMPONENTS

4.1 RMS cow/sheep/poultry/pig ELISA

Each RMS cow/sheep/poultry/pig ELISA kit contains sufficient material for 24 measurements (including controls) of each species.

The following components are provided in each kit:

Component	Detail	Vials / bottle / pack	Ready to use
RMS Control	Positive for cow, pig, sheep and poultry	1	✓
Cow Conjugate		1	✓
Pig Conjugate		1	✓
Sheep Conjugate		1	✓
Poultry Conjugate		1	✓
TMB Substrate		1	✓
Wash solution concentrate		1	10-fold concentrate
Diluent Concentrate Type 9		1	5-fold concentrate
Stop Solution	**CARE**: STRONG ACID	1	✓
Cow / pig / sheep / poultry plate	96 microwells (3 x 8 well strips per species) Red – Cow Yellow – Pig Green – Sheep Blue – Poultry	N/A	✓
Package insert		N/A	N/A
Blank result form		N/A	N/A

4.2 RMS Horse ELISA (Neogen item 902122M)

Each RMS Horse ELISA kit contains sufficient material for 48 measurements (including controls).

The following components are provided in each kit:

Component	Detail	Vials / bottle / pack	Ready to use
RMS Control	Positive for horse	1	✓
Horse Conjugate		1	✓
TMB Substrate		1	✓
Wash solution concentrate		1	10-fold concentrate
Diluent Concentrate Type 9		1	5-fold concentrate
Stop Solution	**CARE**: STRONG ACID	N/A	✓
Horse plate	48 microwells (6 x 8 wells)	N/A	✓
Package insert		N/A	✓
Blank result form		N/A	N/A

5. MATERIALS/EQUIPMENT REQUIRED BUT NOT PROVIDED

Reagents:

- Sodium chloride
- Purified water

Required equipment:

- Miscellaneous laboratory plastic and/or glassware, plate seals, equipment for sample handling/extraction and containers suitable for meat extracts (e.g., Stomacher bags, 10 mL graduated tubes).
- Precision micropipette(s) capable of delivering 50 and 100 µL, plus disposable tips.

Optional equipment (recommended):

- Domestic blender or mincer or Stomacher and suitable sample preparation bags
- Orbital microwell plate shaker set at 700–800 rpm.
- Centrifuge and appropriate centrifuge/microfuge tubes for clarifying sample extract.
- Microwell washer (e.g., NUNC Immuno Wash 8) or wash bottle
- Microwell plate reader, fitted with 450 nm interference filter (calibrate regularly) or spectrophotometer and disposable semi-micro cuvettes.
- Disposable plastic Pasteur pipettes (nominal drop size (water) 35–40 µL) for sample/reagent addition.

6. SAFETY/COSHH NOTE

Good laboratory practice techniques should be employed when using this kit; if such practices are used the reagents constitute a very low potential risk to health. Safety clothing (lab coat, glasses and gloves if necessary) should be worn and skin contact with reagents avoided. Do not ingest. Any contact with skin/eyes should be treated by washing/irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

7. PREPARATION AND EXTRACTION OF SAMPLES

7.1. Preparation of sample extraction buffer

Prepare a saline solution (0.9% (0.15 M) sodium chloride, preferably in purified water) for use in the extraction of meat samples.

7.2. Preparation and extraction of samples/controls

Some samples (e.g., frozen swarf, minced or mechanically deboned meat) may be extracted with no further preparation; larger pieces (e.g., carcass meat or frozen core samples) should be finely chopped, minced, stomached or blended before use. The more finely divided and homogeneous the sample the better the analytical result. Milk should be tested after 1:9 dilution with Working Diluent Solution only; mix well.

In view of the sensitivity of the method, care must be taken at this stage not to cross contaminate samples; any equipment/utensils used must be either disposable or thoroughly washed between extractions.

1. Weigh out 1 g of the prepared meat sample into a clean, 10 mL graduated tube or a clean Stomacher bag.
2. For extraction using a graduated tube, make up to the 10 mL mark with saline solution or water. Stopper and mix thoroughly by hand or on a suitable shaker. **ALTERNATE**: Stomach sample with 9 mL saline or water in Stomacher bag or blend sample to obtain a sample extract. Allow to stand for **10 minutes**.
3. Prepare a 1:9 dilution by adding 0.1 mL (or 1 drop) of the liquid or slurry to 0.9 mL (or 9 drops) of Working Diluent Solution; mix well.

Sample notes:

If, depending on the nature of the material being investigated, a larger sample size is felt to be appropriate, an alternative container will be required. The amount of saline added must be scaled up proportionally (e.g., for a 2.5 g sample use a 25 mL tube and make up to 25 mL with saline).

Depending on the type of sample, a liquid layer may appear above the settled (meat) layer; alternatively, a thin slurry may be obtained.

If the sample has a high fat content, it may be appropriate to carefully remove a portion of the aqueous solution (e.g., using a clean Pasteur-type pipette) into a second, clean container prior to making the 1:9 dilution.

8. ENZYME IMMUNOASSAY

8.1. Preparation

1. Prepare diluted sample extracts (section 7) and kit materials (see below).
2. Remove all reagents from the kit box and allow to reach **room temperature** (18–22°C) before starting the test.
3. RMS Control, Species Conjugates, TMB Substrate and Stop Solution are supplied ready to use. **No preparation** is necessary, simply mix by repeated inversion (do not shake).
4. **Wash solution concentrate:** Supplied as a 10-fold concentrate. Dilute 1:9 in purified water to prepare Working Wash Solution. For example add 100 mL (± 1 mL) to a volumetric flask/cylinder and make up to 1 L (± 10 mL) with purified water.
5. **Diluent concentrate type 9:** Supplied as a 5-fold concentrate. Dilute 1:4 in purified water to prepare Working Diluent Solution. For 96 microwells, use total contents of the bottle (20 mL) by making up to 100 mL with purified water in a volumetric flask/cylinder. For any other number of microwells, dilute 1:4 with water (e.g., for a group of 24 microwells, add 4.5 mL (± 0.2 mL) to 18 mL of purified water).

NOTE: Working Diluent 9 is used for sample dilution and as the negative control in the kit. It is important to ensure that Working Diluent is **not** contaminated during sample preparation.

6. ELISA: Open the foil pouch. Take out the plate, remove the strips not required and return them to the pouch taking care that the desiccant capsule is present; **reseal the pouch carefully**.

NOTE: With a pencil, number the columns in sequence on the upper frosted edge of the strips in use; this preserves the identity of the strips should they become detached from the frame.

8.2. Test procedure

When familiarising oneself with the kit smaller test runs should be performed. One well is used for the RMS control, two for Working Diluent (which is used as a negative control in the test) and the remainder for test samples, which are run singularly. Results may be recorded on the forms provided. For larger test runs refer to worksheet for layout.

NOTE: When testing has been started, all steps should be completed without interruption.

Step	Procedure	Volume	Description
1	Addition	100 µL (2 drops)	Pipette diluted sample extract, RMS Control or working diluent (negative control) into appropriate assay well.
NOTE: Use a separate disposable tip for each pipetting step to avoid cross-contamination.			
2	Mix/incubate		Cover and incubate at room temperature for either **10 minutes** with constant mixing (orbital shaker) or **20 minutes** static.
3	Wash		Wash 3–5 times with Working Wash Solution*.
4	Addition	50 µL (1 drop)	Dispense Species Conjugate into each relevant assay well..
5	Mix/incubate		Cover and incubate at room temperature for either **10 minutes** with constant mixing (orbital shaker) or **20 minutes** static.
6	Wash		Wash 3–5 times with Working Wash Solution*.
7	Addition	100 µL (2 drops)	Dispense TMB Substrate into each assay well.
8	Mix/incubate		Cover and incubate at room temperature for either **10 minute**s with constant mixing (orbital shaker) or mix by hand and leave **10 minutes** static.
Visually assess the microwells for the presence of a blue colour. Samples with a similar or darker blue colour to that of the RMS Control wells are **positive**. Samples with less colour than the RMS Control or no colour are **negative**.			
OR			
9	Addition	50 µL	Dispense acid Stop Solution into each assay well
10	Mix		Mix for **10 seconds (± 1 second)** gently by hand to stop
FOR ABSORBANCE MEASUREMENT USING A PLATE READER:			
11	Read absorbances using a microplate reader fitted with a 450 nm filter, blank the reader on air**. Record results on the worksheet provided.		
FOR ABSORBANCE MEASUREMENT USING A SPECTOPHOTOMETER:			
11	Transfer 100 µL from each well to a cuvette; add 900 µL distilled water to each cuvette. Read absorbances at 450 nm against a water blank. Record results on the worksheet provided.		

***Washing:** Empty the wells by inverting over a sink and, in one rapid movement, flicking out the contents. Using a 500 mL wash bottle containing Working Wash Solution fill each well to the top, allow to stand for **20–30 seconds** then empty the plate as described above: repeat this washing sequence for a total of three washes. Remove residual liquid and bubbles by tapping upside down on several layers of absorbent tissue.

When inverting the plate be sure to squeeze the plastic frame at the centre of the long edges to prevent the strips from falling out of the frame.

Alternatively, at the end of the incubation period aspirate the contents of all wells in column 1 using a microwell washer then fill wells with Working Wash Solution. Complete this sequence for each column of wells (all wells are now filled with Wash Solution). Return the microwell washer to column 1 and repeat the aspiration/fill sequence four times (each column will have been washed five times). Finally, use the microwell washer to aspirate all the wells then tap the plate upside down on several layers of absorbent tissue to remove residual droplets of Wash Solution and bubbles.

****Absorbance measurement:** Using a microplate reader fitted with a 450 nm filter, blank the reader on air. Measure the absorbance of each of the assay wells starting at well "B" in column 1;complete the reading of column 1 before moving to the top of column 2. Repeat this process until all wells have been measured. All readings should be completed within **30 minutes** of adding the Stop Solution. Record the results on the assay well worksheet provided and calculate the mean absorbance values.

9. RESULTS

The **BioKits Raw Species Identification Test Kit** is designed to assist in the qualitative assessment of species content in uncooked meats and meat products. Because of the variations in content and treatment of meat samples both pre- and post-slaughter, the amount of residual albumin in the extract may vary. This must be taken into consideration when interpreting results. If testing to confirm the **presence** of a species (e.g., to prove a sample really is pork), note that a sample containing <10–20% pork (e.g., 80–90% adulterated meat) will give a result similar to that of 100% pork. Samples heated at 70°C remain positive for at least an hour; samples treated at 100°C become negative in **10–20 minutes**. UHT and sterilised milks are **negative** and raw and pasteurised milks are **positive** in the appropriate tests.

10. PERFORMANCE CHARACTERISTICS

The kits are tested at room temperatures of 19–23ºC. Under these conditions, when visually assessed, **Negative Control** wells in each species test should appear virtually colourless, while the **RMS Control** will give a definite blue colouration. Performance of the test above or below these temperatures may necessitate either the reduction or extension (respectively) of incubation times.

Significant visible colour in any of the Negative Control wells may indicate contamination of the **TMB Substrate** or splashing of **Species Conjugate** during addition to adjacent wells or poor washing technique. Colouration of the Negative Control wells or poor agreement between replicate samples/wells is an indication of a problem during the performance of the test and any results from that test should be interpreted with caution.

It is important to note that because of inherent variations in the soluble protein content of meat, the BioKits Raw Species Identification Test Kit is not quantitative, however in our laboratories, diluted lean meat extracts and "spiked" samples give consistent responses with a limit of quantitation of ±1%. More information is available in the product's validation report.

The following information may also be of use in interpreting results. The **RMS Control** is equivalent to a sample containing significant levels of the relevant meat (approximately 1–2% fresh, lean meat); 100% lean meat samples will give significantly more colour in the test. Using the cut off value described below, the test is capable of detecting 1% adulteration of lean meat in the original sample. However, each laboratory should verify its own detection limits for the test, preferably using fresh, lean meat samples. To obtain a solution corresponding to 1% lean meat, a further 1:99 dilution of the saline extract of 100% meat must be made (e.g. take 0.1 mL saline extract and add to 9.9 mL of saline solution in a clean tube). This solution, if further diluted prior to assay (1:9 in Working Assay Diluent) as described on page 5, approximates to a sample extract from a meat adulterated with 1% of the relevant meat. Using this approach or performing spike/recovery experiments, a 1% solution or 1% spiked meat/meat product should consistently give a positive result.

NOTE: Plate reader/spectrophotometer data only: The use of a cut-off value, based on the absorbance values generated by the negative control wells, gives greater sensitivity than visual assessment and enables the classification of low level contamination below the level of the **RMS Control**.

To calculate a cut off value, take the mean absorbance of the negative control wells and multiply by a factor, F. Test samples with an absorbance value **greater than** the cut-off value are significantly higher than the negative control and can be classified as **positive**. If a test sample gives a result very close to the cut-off value, its extraction and/or analysis should be repeated. It is important to note that because the colour of the negative control, and therefore the cut-off value, is related (e.g., to reagent quality, sample type and individual technique the value of the factor, F may vary). As a guide, if a value of at least F=2.5 is achieved, the value of factor F will vary between assay kit, operator and laboratory and ideally should be recalculated for each sample type as described below.

Setting the cut-off value yourself:

If absorbance values obtained from 1% samples are consistently higher than the suggested cut off value, recalculate the factor, F as follows:

$$F = \frac{\text{1\% lean meat absorbance}}{\text{Mean absorbance of negative control wells}}$$

The recalculated factor is used in Section 1 of the results form to determine the new cut off value. If absorbance values obtained from 1% samples are consistently lower than the suggested cut off value, it is suggested that you test samples equivalent (e.g., 2 or 3% meat) to ensure that they are detected using the suggested cut-off; you should also increase your quoted limit of quantitation.

11. EXAMPLE DATA

1. Record visual assessment or absorbances of controls.

	SPECIES							
	Cow STRIP		Pig STRIP		Shp STRIP		Pou STRIP	
	Visual	A450	Visual	A450	Visual	A450	Visual	A450
RMS Control (+)	+++	0.503	+++	0.535	+++	0.558	+++	0.682
Negative Control (-)	-	0.095	-	0.071	-	0.048	-	0.061
Negative Control (-)	-	0.095	-	0.074	-	0.049	-	0.057

Mean Absorbance of the Negative Control wells (C)	0.069
F*	2.5
Cut Off = C x F**	0.172

2. Record visual assessment or absorbance of samples.

	Pou STRIP			Shp STRIP	
Sample ID	**Abs value**	**Cut-off / visual +/-**	**Sample ID**	**Abs value**	**Cut-off / visual +/-**
Turkey sausage T1	1.445	++	Lamb mince T1	2.002	+++
Chicken burger T2	3.528	+++++	Pork burger T2	0.051	-
Barbary duck T3	1.157	++	Veal escalopes T3	0.058	-
Lamb mince T4	0.055	-	Beef sausages T4	0.065	-
Beef burger T5	0.049	-	Breaded chicken T5	0.052	-
Pork sausage T6	0.063	-	Beef mince T6	0.065	-

12. SHELF LIFE

Diluted wash buffer: Once diluted 1:9 the wash buffer is stable at room temperature in a sealed clean container for at least one week.

Diluted assay diluent: Fresh assay diluent should be prepared for each assay.

Extraction buffer: Fresh extraction buffer should be prepared daily.

Extracted samples: The diluted sample extracts may be stored at 2–8°C for up to three days. If prolonged storage is required the extracts must be stored frozen; they are stable for up to five freeze thaw cycles and for at least three months at 20°C.

Kit reagents: The kit should be stored at 2–8ºC. The shelf life of unopened kit components is indicated by the expiry date on the respective labels. Once the kit reagents have been opened, exposure to elevated (i.e., room) temperatures should be minimised.

ELISA: Must be kept dry; if necessary (desiccant turns off-white/cream) the desiccant capsule can be re dried by placing in a 100ºC oven (changes to yellow-orange in colour).

Providing these instructions are complied with the opened kit reagents should be stable for many weeks or months at 2–8ºC.

Neogen Corporation ("Neogen") warrants the product supplied ("the product") against defects in materials and workmanship when used in accordance with the applicable instructions for a period of one year from the date of shipment of the product or if shorter, for a period not to extend beyond a product's printed expiration date. If the customer establishes that the product does not conform to this limited warranty, Neogen shall, at its option, replace such product with a similar product or allow the Customer credit for their invoice value, but Neogen will have no further liability to the Customer. Neogen makes no other warranties expressed or implied, including but not limited to any implied warranties or merchantability or fitness for a particular purpose. Neogen does not warrant against damages or defects arising in shipping or handling, or out of accident, or improper or abnormal use of the product. Neogen shall not be liable for any damages (including special or consequential damages) or expenses arising directly or indirectly from the use of its product.

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