ELISA-TEK®

RAW MEAT SPECIATION KITS

For the Qualitative Detection of Animal Species Content in Uncooked Meat and Meat Products by Enzyme-Linked ImmunoSorbent Assay (ELISA)

INSTRUCTIONS FOR USE

ELISA-TEK® Raw Cow Kit - Catalog # 510511
ELISA-TEK® Raw Pig Kit - Catalog # 510521
ELISA-TEK® Raw Poultry Kit - Catalog # 510531
ELISA-TEK® Raw Sheep Kit - Catalog # 510541
ELISA-TEK® Raw Horse Kit - Catalog # 510551
ELISA-TEK® Raw 3 Species Kit - Catalog # 510503
ELISA-TEK® Raw 4 Species Kit - Catalog # 510504
ELISA-TEK® Raw Custom Kit - Catalog # 510501

ELISA Technologies
Committed to food and feed safety

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RAW MEAT SPECIATION KITS

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RAW MEAT SPECIATION KITS

INTRODUCTION

Preventing adulteration of animal products with less desirable or objectionable species is important for economic, regulatory, health, and cultural reasons. The identification of meat species is performed in many countries to assure consumers that the meat and poultry they purchase is safe, wholesome, unadulterated, and properly labeled, and may be of importance in various communities where the consumption of a particular meat is proscribed.

The ***ELISA-TEK®* RAW MEAT SPECIATION KITS** are based on antibodies raised to species-specific proteins and employ the techniques of the **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay **(ELISA)**. The ***ELISA-TEK®* RAW MEAT SPECIATION KITS** have been formatted and refined for ease-of-use as sensitive and specific tests designed to aid in the resolution of species content in UNCOOKED meat, meat products, and milk.

PRINCIPLE OF THE TEST

***ELISA-TEK®* RAW MEAT SPECIATION KITS** utilize non-competitive, sandwich-type enzyme immunoassays for the positive identification of species content in a sample. Meat samples are minced and then extracted using a simple saline solution; dilutions of this extract are added to plastic microwells, which have been pre-coated with a preparation of purified, species-specific antibody. The species-specific proteins in the diluted meat extract bind to the antibody attached to the well. After allowing the reaction to proceed, any unbound material is removed by aspiration and washing.

The amount of protein remaining bound to the antibody-coated well is determined by the addition of a peroxidase conjugated (species-specific) antibody. After incubation, excess conjugate is removed by aspiration and washing. Bound peroxidase activity is determined by adding a fixed amount of TMB substrate, which develops a blue coloration in the presence of peroxidase. Stopping the color development with 25% H_3PO_4 causes the reaction to turn yellow. Color development is directly proportional to the original amount of specific protein in the extract and a QUALITATIVE estimate of meat species type may be made by using a spectrophotometer or plate reader. A summary flow chart of the enzyme immunoassay procedure is given on page 9 of this manual.

SAFETY/COSHH NOTE

The techniques of “Good Laboratory Practice” should be employed when using this kit; if such practices are used, then 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 would be treated by washing/irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

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RAW MEAT SPECIATION KITS

KIT COMPONENTS

NOTE: *These kits are sold in a range of test combinations containing one or more sets of species-specific reagents. Exact kit contents will therefore vary depending on the type of kit received.*

A. **ONE ANTIBODY-COATED MICROWELL MODULE** comprised of twelve single column strips of eight microwells each (96 test wells total), held in a plastic frame and packed in a laminate pouch with desiccants. The interior of each microwell has been coated with a calibrated amount of species-specific antibody and dried, with each strip labeled according to its specificity.

COW - Beef PIG - Pork POU - Poultry SHP – Sheep/Goat HRS - Horse

B. **TWO or MORE** vials of **RAW CONTROL** containing 2.0 mL each of species-specific control in buffered solution with carrier protein and 0.04% sodium azide as preservative. Each serves as a positive control in the appropriate test, and can be used as a negative control in any other Raw Meat Species test. However, *use of the appropriate lean tissue extracts as positive and negative controls is recommended.*

NOTE: *The RAW CONTROLS are intended to produce a positive response for the species indicated without further dilution. The RAW CONTROLS are NOT EQUIVALENT to the preferred 100% TISSUE POSITIVE CONTROLS (see page 7) and must NOT be used for preparation of 1% TISSUE POSITIVE CONTROLS.*

C. **THREE or MORE** vials of **SPECIES-SPECIFIC PEROXIDASE CONJUGATE** containing 3.6 mL of the relevant conjugated antibody(s) in a buffered solution with a stabilizer.

D. **ONE** vial of **ASSAY DILUENT CONCENTRATE** containing 20 mL of a five-fold (5×) concentrate of Tris-buffered saline with Tween® 80 detergent.

E. **ONE** vial of **TMB SUBSTRATE** containing 12.0 mL buffered and stabilized TMB.

F. **ONE** vial of **STOP SOLUTION** containing 12.0 mL of 25% w/v phosphoric acid (H_3PO_4) in deionized water.

G. **ONE** bottle of **WASH SOLUTION CONCENTRATE** containing 100 mL of a ten-fold (10×) concentrate of Tris-buffered saline with a wetting agent.

H. **ONE INSTRUCTIONS FOR USE** manual**,** with one **BLANK WORKSHEET** and **RESULTS FORM**.

SHELF LIFE

The shelf life of the unopened kit is indicated on the outside label. Individual component shelf lives may vary as indicated on the respective labels. Exposure of the kit and kit components to ambient or elevated temperatures (>2-8°C) should be minimized.

KIT STORAGE INSTRUCTIONS

***ELISA-TEK*® RAW MEAT SPECIATION KITS** should be stored at 2-8°C (refrigerated). DO NOT FREEZE. Kit components should be removed from refrigeration and brought to room temperature (~20-25°C) before beginning the assay. Return unused components to refrigeration (2-8°C) after use.

The **ANTIBODY-COATED MICROWELL MODULE** must be kept **DRY and WELL SEALED.** If the desiccant packet turns pink, it can be dehydrated by placing in a 100°C oven until the desiccant changes to dark blue in color. Alternately, the desiccant can be replaced or the microwell module may be stored in a desiccation chamber at 2-8°C (refrigerated).

EXTRACTED SAMPLE STORAGE

In-house single lab validation testing indicates 100% and 1% positive tissue sample extracts were stable following 3-6 freeze thaw cycles or 4°C storage for 3-7 days. For optimal results it is recommended that sample extracts be tested fresh on the day of extraction and repeated freeze-thaw cycles be avoided.

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents: Purified water, Sodium chloride.

Equipment: Centrifuge capable of 10,000 ×g and appropriate centrifuge tubes (alternately, the sample extract may be filtered using Whatman #4 or similar filter paper).

Miscellaneous laboratory plastic and/or glassware, including measuring cylinders, pipettes, knives, and containers suitable for meat extracts.

Precision micropipette (e.g., Eppendorf 2233351, or equivalent) and tips capable of delivering 25, 50, 100 and 1000 microliter volumes.

Microwell plate reader, dual wavelength, fitted with 450 nm and 630 nm filters.

Optional equipment:

Stomacher and stomacher bags or Whirl-Pak® bags. Alternately, a domestic blender or mincer may be used.

Precision repeating pipette (e.g., Eppendorf 22260006) and tips capable of delivering 100 microliter volumes.

Precision multichannel pipette and tips capable of delivering 100 microliter volumes.

Microwell washer (e.g., Nunc Immuno Wash 8, Biotek EL 403, etc.) or, alternately, a Reagent Wash bottle may be used.

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PROCEDURAL NOTES AND PRECAUTIONS

1. Review the complete Instructions for Use before performing the Raw Meat Species ELISA.

2. ***ELISA-TEK*® RAW MEAT SPECIATION KITS** are intended to be used as an integral unit. The components have been calibrated and optimized to produce consistent results. Components from other kits and/or lots should not be interchanged as they may alter the precision of the assay.

3. Each microwell strip may be used only once.

4. It is not necessary to perform the immunoassay under sterile conditions.

5. All components and test specimens should be at ambient temperature (~20-25°C) before testing begins.

6. Mix all reagents and test specimens thoroughly before use by gentle repeated inversions or swirling. DO NOT SHAKE.

7. When testing has started, all steps should be completed without interruption.

8. Care must be taken to not cross-contaminate wells. A new pipette tip must be used for each sample and control. Do not touch the top of the wells with your fingers or pipette tips.

9. Do NOT allow the conjugate to mix with the substrate. If plastic troughs are used to disperse conjugate and substrate solutions ensure that they are always kept separate.

10. The knife, cutting surface, and hands must be thoroughly cleaned and rinsed between samples and controls to avoid cross-contamination.

11. Incomplete well washing will adversely affect the outcome.

12. All samples to be tested must be raw or uncooked.

13. It is advisable to number each strip/column with a pencil on the upper frosted edge of the strip. This preserves the identity of the strips should they become detached from the frame.

14. As stated previously, the use of lean muscle tissue extract from the appropriate species is recommended for both positive and negative controls. The RAW CONTROLS are provided as indicators of proper assay procedure only.

SAMPLE PREPARATION AND EXTRACTION

Extraction solution:

Prepare a saline solution (0.9% w/v sodium chloride in deionized water, e.g., 9 g NaCl in 1 L H_2O) for use in the extraction of meat samples. If saline is unavailable, deionized water may be used as an alternative extractant.

Preparation of test samples:

Some samples (ground, minced, or mechanically de-boned meat) can be extracted with no further preparation. Larger pieces of meat or frozen core samples should be finely chopped, minced, blended, or stomached before use. The more finely divided and homogeneous the sample, the better the analytical result.

Extraction of test samples:

> ***NOTE:*** *Due to the sensitivity of the method, care must be taken at this stage to prevent cross-contamination of samples. Any equipment, utensils, containers, or surfaces used must be thoroughly washed or discarded between extractions.*

1. Weigh out 1 gram of the diced (minced, etc.) sample into a clean stomacher bag or Whirl-Pak® bag, tube, or beaker.

2. Add 9 mL of the prepared saline solution (0.9% w/v NaCl).

> ***NOTE:*** *If, depending on the nature of the material being tested, a larger sample size is felt to be appropriate, an alternative container may be required. If a larger sample size is used, the amount of saline used must maintain the ratio of material to solvent (e.g., for a 5 gram sample use 45mL of saline solution).*

3. Mix the contents; e.g., place the bag and contents into a stomacher for 10 seconds. Alternatively, stopper and mix the tube, or mechanically disrupt the solution in the beaker.

4. Leave undisturbed for 10 minutes at room temperature.

> ***NOTE:*** *Depending on the type of sample, a clear liquid may appear above the settled (meat) layer; alternatively, a thin slurry may be obtained. If necessary, clarify the extract solution by filtration (Whatman filter paper or a 0.45 µm filter may be used) or centrifugation (10,000 ×g for 10 min). 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 pipet into a clean container) prior to making the 10× dilution.*

5. Prepare a 10× dilution of extraction supernatant by adding 100 µL of the clear liquid or slurry to 900 µL of Working Assay Diluent; mix well.

6. The diluted sample extract is now ready for the meat species enzyme immunoassay.

PREPARATION OF SPECIES TISSUE CONTROLS

NOTE: *Positive and Negative RAW CONTROLS are provided "ready-to-use" with each kit and require no additional dilution. Each species control may be used as a positive control for a homologous (same species) test and as a negative control in any heterologous (other species) test.*

NOTE: *The RAW CONTROLS provided with the kit are NOT EQUIVALENT to 100% TISSUE POSITIVE CONTROLS and should not be used for preparation of 1% TISSUE POSITIVE CONTROLS. TISSUE CONTROLS are therefore the most appropriate controls for use in these assays.*

NOTE: *Care must be taken not to cross contaminate meats used for preparation of TISSUE CONTROLS. Meat used for preparation of TISSUE CONTROLS must not come into contact with meat or with surfaces that have been in contact with any other meats.*

Preparation of 100% raw species tissue controls:

1. Prepare a portion of lean, raw meat by dicing, mincing, blending, or finely chopping.

2. Weigh 5 grams of the diced tissue in a stomacher or Whirl-Pak® bag. Add 45 mL of normal saline (0.9% Sodium chloride).

3. Place bag and contents into a stomacher for 10 seconds.

4. Remove from the stomacher and leave undisturbed for 10 minutes at room temperature.

5. If necessary, clarify the extract solution by filtration or centrifugation.

6. Transfer the solution to a clean, properly labeled vial (e.g., Raw Pig Tissue Control, preparation date).

7. **This extract must be further diluted 10× in Working Assay Diluent to be ready to use as the 100% control in the Raw Meat Species ELISA**.

Preparation of 1% positive raw species tissue controls:

NOTE: *ELISA-TEK® Raw Meat Speciation Kits are formatted so samples containing adulterant species antigens in amounts considered significant by industry regulation (usually approximately 1% adulteration) will produce a visually distinct blue coloration. In order to differentiate samples containing variable amounts of adulterant species as to their probable regulatory status, the use of a 1% positive tissue control is recommended.*

1. Select a POSITIVE RAW SPECIES TISSUE CONTROL of the appropriate species (prepared as above at step 6, not yet diluted in working diluent) for the test you wish to perform.

2. Dilute 50 µL of this control to 5.00 mL with saline solution (or alternatively dilute with the raw tissue extract of an appropriate (negative) species) to make a 1% extract solution.

3. **This 1% control solution must be further diluted 10× in Working Assay Diluent to be ready to use as the 1% control in the Raw Meat Species ELISA**.

PREPARATION OF KIT MATERIALS

A. ANTIBODY COATED MICROWELL MODULE: Open the foil pouch. Referring to your plate plan, select the desired number of strips for each named species and fit into a spare frame. Replace the remaining frame and strips in the pouch, taking care that the desiccant is present, and reseal the pouch carefully.

B. RAW CONTROLS: No preparation necessary.

C. SPECIES-SPECIFIC PEROXIDASE CONJUGATES: No preparation necessary.

D. ASSAY DILUENT CONCENTRATE: ASSAY DILUENT CONCENTRATE is supplied as a 5× concentrate and requires dilution 1:5 in distilled/deionized water to prepare WORKING ASSAY DILUENT (e.g., mix the contents of the bottle (20 mL) with 80 mL H_2O). This diluted reagent is used for the final 10× dilution of meat extracts and test samples.

E. TMB SUBSTRATE: No preparation necessary.

F. STOP SOLUTION: No preparation necessary.

G. WASH SOLUTION CONCENTRATE: WASH SOLUTION CONCENTRATE is supplied as a 10-fold concentrate and requires dilution 10× in distilled/deionized water to prepare WORKING WASH SOLUTION.

For 96 test wells, add the total contents of the WASH SOLUTION CONCENTRATE (100 mL) to 900 mL of distilled or deionized water and mix gently by inversion.

For a smaller number of test wells, dilute the WASH SOLUTION CONCENTRATE 10× in distilled/deionized water (e.g., for a group of 24 test wells, add 24 mL of WASH SOLUTION CONCENTRATE to 216 mL of water).

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ENZYME IMMUNOASSAY PROCEDURE: SUMMARY FLOW CHART

PROCEDURE	VOLUME	TIME	DESCRIPTION
Addition	100 µL	--	Pipette WORKING ASSAY DILUENT, CONTROLS, and DILUTED SAMPLE EXTRACTS into respective test wells
Incubate	--	20 min	Incubate at room temperature
Wash	--	--	Wash each well 3 times using WORKING WASH SOLUTION
Addition	100 µL	--	Pipette SPECIES-SPECIFIC PEROXIDASE CONJUGATE into all test wells
Incubate	--	20 min	Incubate at room temperature
Wash	--	--	Wash each well 5 times using WORKING WASH SOLUTION
Addition	100 µL	--	Pipette TMB SUBSTRATE SOLUTION into all test wells
Incubate	--	10 min	Incubate at room temperature
Addition	100 µL	--	Pipette STOP SOLUTION into all test wells and mix by gently rotating the microplate
Results	--	--	Measure the absorbance of each well at 450-630 nm DUAL WAVELENGTH using a microplate reader and perform data analysis

DETAILED ENZYME IMMUNOASSAY PROCEDURE

Plate Layout Plan:

Each ***ELISA-TEK®*** **RAW MEAT SPECIATION KIT** can be used as a 96-well unit or may be divided into a variety of strip formats depending on the number of samples to be analyzed and the kit type (i.e., species combination). IT IS IMPORTANT to prepare a test layout showing the wells you will use for controls and samples. This layout plan will be used to determine the number of strips of each species you will need to use, the locations for samples, controls, and species specific reagents during the procedure, and to locate and identify the data/result for each control and sample.

> ***NOTE:*** *It is recommended when first familiarizing oneself with the kit that smaller test runs be performed. All reaction wells are run singly and the results may be recorded on the worksheet form provided. For screening samples, single or duplicate microwells for each control and sample extract may be adequate. Regulatory protocols may require use of quadruplicate microwells for each control and sample extract.*

1. Locate the enclosed worksheet template showing the 96-well layout. Determine the number of replicate wells you wish to use for each control and extract. Mark the location of the wells selected for each control and sample extract on the template diagram.

2. Decide the number and type of controls you wish to use for each species you are testing for. Always use at least a positive and a negative RAW CONTROL or TISSUE CONTROL. Ideally each assay will include a 100% POSITIVE TISSUE CONTROL, a 1% POSITIVE TISSUE CONTROL, and one or more NEGATIVE TISSUE CONTROLS. For the 100% POSITIVE CONTROL (and the 1% POSITIVE TISSUE CONTROL) use an extract or control of the species being tested for; e.g., if testing for poultry, use chicken as the positive and for the diluted 1% positive control. For the NEGATIVE CONTROL, use one or more species not being tested for (e.g., if the test is for beef, then pork, chicken, etc. would be appropriate negative controls).

Detailed Immunoassay Procedure:

1. Remove your ***ELISA-TEK®*** **RAW MEAT SPECIATION KIT** from the refrigerator. Remove the reagents from the box and allow them to reach room temperature before starting the test.

2. Remove your prepared controls and meat sample diluted extracts (pages 6 and 7) from the refrigerator. Allow them to reach room temperature before starting the test.

3. Remove the MICROWELL MODULE from its pouch and, referring to your plate plan, select the desired number of strips for each named species and fit into a spare frame. Replace the remaining frame and strips in the pouch, taking care that the desiccant is present, and reseal the pouch carefully. Using a pencil, number the strips in sequence on the upper frosted edge; this preserves the identity of the strips should they become detached from the frame.

4. Prepare the necessary kit materials (see page 8).

5. Using a precision micropipette, add 100 µL of WORKING ASSAY DILUENT into each of the wells selected as blanks.

6. Add 100 µL of each DILUTED NEGATIVE TISSUE CONTROL (or NEGATIVE RAW CONTROL) to each of the selected wells.

7. Add 100 µL of DILUTED 1% POSITIVE TISSUE CONTROL to the selected wells.

8. Add 100 µL of the DILUTED 100% POSITIVE TISSUE CONTROL (or POSITIVE RAW CONTROL) in each of the selected wells. [Note: the POSITIVE RAW CONTROL should be added full strength to the wells; it does NOT require dilution prior to assay].

9. Add 100 µL of each DILUTED SAMPLE EXTRACT to each of the selected wells.

10. Mix the plate gently by hand; cover. Allow to stand at room temperature for 20 minutes.

11. At the end of the incubation period, empty the wells by flicking into a sink. Then carefully fill all wells with WORKING WASH SOLUTION using a reagent wash bottle. Repeat this emptying and filling twice more and then empty all wells into a sink. Alternately, place the plate on the carrier of the microplate washer, (or individual strips for a strip washer) which has been primed with WORKING WASH SOLUTION and set to deliver 300 µL per well. Wash and aspirate all wells 3 times. Invert the emptied/aspirated plate and rap sharply several times onto a soft paper towel placed on the lab bench.

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

12. Add 100 µL of SPECIES-SPECIFIC PEROXIDASE CONJUGATE to each well on the relevant (same species) ANTIBODY COATED STRIP(S). Work from the top to bottom of each strip in the sequence. Next, using a fresh pipette/tip, repeat conjugate additions as necessary for each species being run.

13. Mix the plate gently by hand; cover. Allow to stand at room temperature for 20 minutes.

14. At the end of the incubation period, repeat the relevant washing sequence as described in Step 11 above, but perform a total of 5 wash cycles.

15. Add 100 µL of TMB SUBSTRATE SOLUTION to all wells. Work from the top to bottom of each strip in the sequence.

16. Mix the plate gently by hand; cover. Allow to stand at room temperature for 10 minutes.

17. Add 100 µL of STOP SOLUTION to all wells. Work from the top to bottom of each strip in the sequence.

18. Mix the plate gently by hand to distribute the STOP SOLUTION and prevent further color development.

19. Read the plate using a microplate reader equipped with 450 nm and 630 nm filters. Collect the dual wavelength absorbances at 450-630 nm. Read the plate within 10 minutes of adding stop solution.

DETERMINATION OF RESULTS

Instrumental determination of test validity

1. Program your microplate reader to read absorbance at 450-630 nm dual wavelength.

2. Place the microplate on the reader carriage and blank the instrument on the selected blank (diluent) wells (alternatively, read all raw absorbances and manually subtract the average diluent blank O.D. value from each control and sample average O.D. value after step 4).

3. Obtain a printed copy of the absorbance values for each well.

4. Determine the mean absorbance value of each of the 100% POSITIVE TISSUE CONTROL, 1% POSITIVE TISSUE CONTROL, and NEGATIVE TISSUE CONTROL wells.

5. Determine the standard deviation of the replicates of each of the controls.

The assay may be considered **VALID** if:

a. The mean blank-corrected O.D. of the 100% POSITIVE TISSUE CONTROL is greater than 0.600

AND

b. The mean blank-corrected O.D. of the 1% POSITIVE TISSUE CONTROL is greater than 0.250

AND

c. The mean blank-corrected O.D. of each NEGATIVE TISSUE CONTROL is less than or equal to 0.150

If these conditions are not met, the assay is **INVALID** and should be repeated.

If the assay is valid, then samples may be classified as positive or negative as described below:

Instrumental determination of sample status

a. Test samples may be classified as POSITIVE if their mean blank-corrected O.D. value is greater than 0.150 and the assay is valid according to the criteria listed above.

b. Test samples are considered NEGATIVE if their mean blank-corrected O.D. value is less than or equal to 0.150 and the assay is valid according to the criteria listed above.

PERFORMANCE CHARACTERISTICS

***ELISA-TEK*® RAW MEAT SPECIATION KITS**, when used as directed, will qualitatively identify meat and poultry samples containing the species tested for at levels of approximately 1% or greater.

In our laboratories, uncooked, lean meat samples prepared as directed on page 7 (Preparation of Species Tissue Controls) gave positive responses when diluted 100× in negative meat extracts (i.e., an approximation of a sample containing 1% of the meat of interest). Furthermore, composite lean skeletal muscle samples mixed w/w prior to extraction (e.g., 1 g of pork mixed into 99 g of lamb) are recognized as strong positives.

It is important to note that although color development is proportional to the amount of antigen present, ***ELISA-TEK*® RAW MEAT SPECIATION KITS** ARE NOT intended for use as QUANTITATIVE assays. Variations in sample content (e.g., % lean tissue, % moisture, % fat, etc.) and variations in sample treatment must be taken into consideration when interpreting results since the actual amount of antigen present and the amount of adulteration required to produce a positive identification will vary.

***ELISA-TEK*® RAW MEAT SPECIATION KITS** are designed to give optimum performance at room temperature (~20-25°C). Performance of the test under suboptimal conditions is not recommended.

When the kit and operator perform properly, the NEGATIVE CONTROL wells in each species test should appear virtually colorless to the naked eye while the POSITIVE CONTROL in each test will give a distinct blue coloration (prior to the addition of STOP SOLUTION).

Significant visible color (O.D. @ 450-630 nm > 0.250) in any of the blank or negative control wells may indicate contamination of the TMB SOLUTION or splashing of the PEROXIDASE CONJUGATE during addition to adjacent wells. Such coloration of the negative control wells is an indication of a problem during the performance of the test and any results from that test should be interpreted with caution.

SPECIFICITY

Each set of species-specific reagents has been tested against a panel of meat samples (including, where appropriate beef, pork, horse, chicken, duck, goat, sheep and turkey) for cross-reaction and have been found to produce negative responses to the heterologous species samples.

In addition, the tests may respond in the presence of eggs, milk/milk powder, etc. in a particular sample since these products also contain the target species-specific proteins.

A table of known reactivity of various tissue extracts in the raw meat species ELISA is found on the next page.

CROSS REACTIVITY / INTERFERENCES OF VARIOUS TISSUE EXTRACTS

	COW KIT	PIG KIT	POULTRY KIT	SHEEP KIT	HORSE KIT
COW	+++++	-	-	-	-
PIG	-	+++++	-	-	-
CHICKEN	-	-	+++++	-	-
SHEEP	-	-	-	+++++	-
HORSE	-	-	-	-	+++++
DEER*	++	-	-	-	-
COW'S MILK	++++	-	-	-	-
SHEEP'S MILK	-	-	-	++++	-
GOAT	-	-	-	+++	-
GOAT'S MILK	-	-	-	+++	-
TURKEY	-	-	+++	-	-
DUCK	-	-	+++	-	-
EGG**	-	-	++++	-	-

*North American White Tail Deer

**Chicken Egg (albumin and yolk)

"-" = Negative response to 100% lean skeletal muscle sample

"+++++" = Maximum Reaction "+++" = Medium Reaction

Limit of Detection: < 1% for meat (all species)
< 1% for milk (cow or sheep)

DISCLAIMER:

ELISA Technologies, Inc. ensures that its products are made from high quality raw materials but can make no warranty, expressed or implied, as to their suitability other than to qualitatively detect raw meat species antigen content when used exactly in accordance with these instructions.

Reminders are included as to the safe handling of materials and reagents, proper storage of material and reagents, as well as to use universal laboratory safety protocols and procedures.

Use of the kit for any other purpose is considered outside its intended use.

Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to replacement value of the kit at ELISA Technologies, Inc. discretion.

REVISION NOTES

150504: Edited for clarification. Updated kit shelf life and cross-reaction chart. Added revision notes.

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