ELISA-TEK®

MICROWELL KITS

RAW MEAT SPECIATION KITS

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

INSTRUCTIONS FOR USE

SIGMA-ALDRICH®

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800)521-8956 Fax: (800)325-5052
Sigma-Aldrich.com

Manufactured By:
ELISA Technologies, Inc.
2501 NW 66th Court
Gainesville, FL 32653 USA
Tel: (352) 337-3929 Fax: (352) 337-3928
www.elisa-tek.com

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INTRODUCTION

Preventing adulteration of meat foods with less desirable or objectionable meat 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 serum proteins and employ the techniques of the **Enzyme-Linked ImmunoSorbent Assay (ELISA)**. The ***ELISA-TEK® Raw Meat Speciation Kits*** have been formatted and refined for ease-of-use and are 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** are direct, non-competitive (sandwich type) enzyme immunoassays. 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 to serum albumin. Increased concentrations of albumin in the diluted meat extract result in increased albumin binding to antibody attached to the well. After allowing the reaction to proceed any unbound material is removed by aspiration and washing.

The amount of albumin remaining bound to the antibody coated well is determined by reaction with a fixed amount of 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 yellow coloration in the presence of peroxidase. Stopping the color development with 25% H_3PO_4 causes the reaction to turn blue. Color development is directly proportional to the original amount of albumin 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 6 of this manual.

SAFETY/COSHH NOTE:

The techniques of “Good Laboratory Practice” 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 would be treated by washing/irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

KIT COMPONENTS

A. **ONE ANTIBODY COATED MICROWELL MODULE** is comprised of twelve single column strips of eight microwells (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, dried, and labeled according to its specificity.

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

B. TWO vials of **ALBUMIN CONTROL** containing 2.0mL each of species albumin in buffered solution with carrier protein and 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 ALBUMIN CONTROLS are intended to produce a positive response for the species indicated without further dilution. The ALBUMIN CONTROLS are NOT EQUIVALENT to the preferred 100% TISSUE POSITIVE CONTROLS (see page 8) and must NOT be used for preparation of 1% TISSUE POSITIVE CONTROLS.

C. **THREE** vials of **PEROXIDASE CONJUGATED ANTI-ALBUMIN ANTIBODY** containing 3.6mL of the relevant conjugated antibody(s) in a buffered solution with a stabilizer.

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

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

F. **ONE** vial of **STOP SOLUTION** containing 12.0mL of 25% w/v H_3PO_4 in distilled water.

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

H. **ONE PACKAGE INSERT,** with one **BLANK WORKSHEET** and **RESULTS FORM.**

SHELF LIFE:

The shelf life of the unopened kit components is indicated by the expiration date on the respective labels. Once the kit reagents have been opened, exposure to elevated (i.e., room) temperatures should be minimized. Provided the storage instructions are complied with, the opened kit reagents should be stable for 1 year at 2-8°C.

Note: *Stability characterization indicates the anti-cow albumin peroxidase conjugates are stable for only 6 months, whereas all other kit components were deemed stable for over 1 year when stored at 2-8°C.*

KIT STORAGE INSTRUCTIONS

***ELISA-TEK®* RAW MEAT SPECIATION KITS** should be stored at 2-8°C. The Antibody Coated Microwell module must be kept DRY and WELL SEALED**.** If necessary, the desiccant can be replaced or the microwell module may be stored in a desiccation chamber at 2-8°C. Kit components should be removed from refrigeration and brought to ambient temperature (20-25°C) before beginning the assay. Return unused components to refrigeration (2-8°C) after use.

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: Sodium Chloride

Equipment: Centrifuge capable of 15,000 X *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 and tips capable of delivering 25, 50 and 100 microliter volumes.

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

Optional equipment:

Stomacher and stomacher bags or whirl-pak bags (alternately, a domestic blender or mincer may be used).

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

Precision multichannel pipetter, and tips capable of delivering 100 microliter volumes.

Microwell washer or alternately, a Reagent Wash bottle may be used.

ENZYME IMMUNOASSAY PROCEDURE: SUMMARY FLOW CHART

PROCEDURE	VOLUME	TIME	DESCRIPTION
Addition	100µL	--	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	100µL	--	Pipette ANTI-ALBUMIN PEROXIDASE CONJUGATE into all test wells
Incubate	--	20 min	Incubate at room temperature
Wash	--	--	Wash each well 5 times using WORKING WASH SOLUTION
Addition	100µL	--	Pipette TMB SUBSTRATE SOLUTION into all test wells
Incubate	--	10 min	Incubate at room temperature
Addition	100µL	--	Pipette STOP SOLUTION into all test wells and mix by gently rotating the microplate
Results	--	--	Measure the absorbance of each well at 450-630nm DUAL WAVELENGTH using a microplate reader. Evaluate data.

SAMPLE PREPARATION AND EXTRACTION

Extraction solution:

Prepare a saline solution (0.9% Sodium Chloride in deionized water, e.g., 9g NaCl in 1L H_20) for use in the extraction of meat samples. If necessary, however, water can be used as an 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:In view of 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 9mL of normal saline solution (0.9% 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 be scaled up proportionally (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 or centrifugation. 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 pipette into a clean container) prior to making the 1:10 dilution.*

5. Prepare a 10-fold dilution of extraction supernatant by adding 0.1mL of the clear liquid or slurry to 0.9mL 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 Species Albumin 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 ALBUMIN POSITIVE CONTROLS provided with your 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 45mL 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 1:10 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® 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 microliters 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 1:10 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 SENSITIZED MICROWELL MODULE: Open the foil pouch (label side up) by cutting along the inside of the crimp seal. Remove the MICROWELL MODULE, keeping the wells open side up. 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 with plastic adhesive tape or (preferably) heat sealing.

B. ALBUMIN CONTROLS: No preparation necessary.

C. ANTI-ALBUMIN PEROXIDASE CONJUGATES: No preparation necessary.

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

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

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

PROCEDURAL NOTES AND PRECAUTIONS

1. Review the complete instructions 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. Microwell strips 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 (21-25°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 POSITIVE ALBUMIN CONTROLS are provided as indicators of proper assay procedure only.

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. See also page 16, Plate Layout Plan.*

1) Locate one of the enclosed worksheet templates 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 albumin 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; i.e., 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 kit is for beef, then pork, chicken, etc. would be an appropriate negative).

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 7 and 8) from the refrigerator. Allow them to reach room temperature before starting the test.

3. Open the foil pouch (label side up) by tearing at the notch. Remove the MICROWELL MODULE, keeping the wells open side up. Select the desired number strips for each named species and fit into a spare frame. Replace the remaining frame and strips in the pouch, taking care to replace desiccant, and reseal the pouch.

4. Prepare the necessary kit materials (see page 10).

5. Place 100µL of WORKING ASSAY DILUENT into each of the selected wells.

6. Place 100µL of each DILUTED NEGATIVE TISSUE CONTROL (or NEGATIVE ALBUMIN CONTROL) into each of the selected wells.

7. Place 100µL of DILUTED 1% POSITIVE TISSUE CONTROL in the selected wells.

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

9. Place 100µL of each DILUTED SAMPLE EXTRACT in each of the selected wells.

10. Mix the plate gently by hand, cover, and 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, then empty all wells and dry by tapping the plate upside down on several layers of absorbent tissue to remove residual droplets/bubbles of Working Wash Solution. 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 300µL per well. Wash and aspirate all wells 3 times.

    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 microliters of ANTI-ALBUMIN PEROXIDASE CONJUGATE to each microwell on the relevant (same species) ANTIBODY SENSITIZED STRIP. 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, and 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 10 above, but use a total of 5 wash cycles.

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

16. Mix the plate gently by hand, cover, and allow to stand at room temperature for 10 minutes.

17. Add 100 microliters of STOP SOLUTION to all microwells. 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 450nm and 630nm filters. Collect the dual wavelength absorbances at 450-630nm. 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 450-630nm 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 POSITIVE CONTROL, 1% POSITIVE TISSUE CONTROL, and NEGATIVE 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-subtracted O.D. of the 100% Positive Tissue Control is greater than 0.600 AND

b. The mean blank-subtracted O.D. of the 1% Positive Tissue Control is greater than 0.250 AND

c. The mean blank-subtracted O.D.s of all Negative Controls are less than 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-subtracted 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-subtracted O.D. value is less than 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 8 (Preparation of Controls) gave positive responses when diluted 1:100 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 optimal performance at room temperatures of between 20-23°C. Performance of the test above or below these temperatures may necessitate either a reduction or extension (respectively) of incubation times in order to achieve the desired results.

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 (abs @ 450-630nm > 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 all dairy products contain small amounts of the relevant species Serum Albumin protein.

A table of reactivity of various tissue extracts in the raw meat species ELISA is found on the next page. Further details of the specificity/cross reactivity of the ELISA-TEK® raw meat species kits are available on request.

CROSS REACTIVITY / INTERFERENCES OF VARIOUS TISSUE EXTRACTS

	COW KIT	PIG KIT	POULTRY KIT	SHEEP KIT	HORSE KIT
COW	+++++	-	-	-	-
COW'S MILK	++++	-	-	-	-
BUFFALO	+++	-	-	-	-
HORSE	-	-	-	-	+++++
DONKEY		-	-	-	++++
PIG	-	+++++	-	-	-
SHEEP	-	-	-	+++++	-
SHEEP'S MILK	-	-	-	++++	-
GOAT	-	-	-	+++	-
GOAT'S MILK	-	-	-	+++	-
TURKEY	-	-	+++	-	-
CHICKEN	-	-	+++++	-	-
DUCK	-	-	+++	-	-
EGG (CHK WHITE/ YOLK)	-	-	++++	-	-

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

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

Limit of Detection < 1% for meat (all species)
< 5% for milk (relevant species only)

ASSAY: Raw Pork **OPERATOR:** DCM **DATE:** 07-20-11

CONTROL or SAMPLE ID
1) Gr. Beef #1
2) Gr. Beef #2
3) Gr. Beef #3
4) Gr. Beef #4
5) Gr. Beef #5
6) Gr. Beef #6
7) Gr. Beef #7
8) Gr. Beef #8
9) Gr. Beef #9
10) Gr. Beef #10
11)
12)
13)
14)
15)
16)
17)
18)
19)
20)
21)
22)
23)
24)
25)

PLATE LAYOUT PLAN:

◁ 1) Identify & Record Controls and Samples

▽ 2) Mark Species of Each Strip to be Used:

SPECIES	PK	PK	PK	PK	PK	PK	PK	PK	--	--	--	--
STRIP #	1	2	3	4	5	6	7	8	9	10	11	12
A	Dil	Bf	Ch	#1	#3	#5	#7	#9				
B	Dil	Bf	Ch	#1	#3	#5	#7	#9				
C	Dil	Bf	Ch	#1	#3	#5	#7	#9				
D	Dil	Bf	Ch	#1	#3	#5	#7	#9				
E	1% Pk	Pk	Sh	#2	#4	#6	#8	#10				
F	1% Pk	Pk	Sh	#2	#4	#6	#8	#10				
G	1% Pk	Pk	Sh	#2	#4	#6	#8	#10				
H	1% Pk	Pk	Sh	#2	#4	#6	#8	#10				

△ 3) Plot Location of Controls and Samples:

PROCEDURE RECORD:

▽ 4) Record Procedure Times and Temps:

Step #	Procedure	Volume to Add	Incubate For	Time Added	Temp. (Room)
4 - 7	Add Controls and Samples	100 µL	20 min.	10:18	22.5
9	Add Anti-Species Conjugates	100 µL	20 min.	10:42	22.5
12	Add TMB Substrate	100 µL	10 min.	11:07	23.0
19	Add Stop Solution	100 µL		Read & Record	23.0

ASSAY: Raw Pork **DATE:** 07-20-11

CONTROL SPECIES	CONTROL ID or LOT #	SPECIES TESTED	MEAN ABSORBANCE (blank subtracted)	STANDARD DEVIATION	VALID or INVALID 100% Positive > 0.600 1% Positive > 0.250
Diluent	(Dil)	Pork	0.044	0.002	-
1% Pork	(1%)	Pork	0.427	0.002	+
Beef	(Bf)	Pork	0.037	0.001	-
Pork	(Pk)	Pork	1.065	0.014	+
Chicken	(Ch)	Pork	0.043	0.002	-
Sheep	(Sh)	Pork	0.050	0.001	-
SAMPLE IDENTIFICATION		SPECIES TESTED	MEAN ABSORBANCE (blank subtracted)	STANDARD DEVIATION	SAMPLE RESULT Positive = >0.150
1) Gr. Beef #1		Pork	0.066	0.001	-
2) Gr. Beef #2		Pork	0.046	0.001	-
3) Gr. Beef #3		Pork	0.159	0.002	+
4) Gr. Beef #4		Pork	0.046	0.001	-
5) Gr. Beef #5		Pork	0.694	0.020	+
6) Gr. Beef #6		Pork	0.058	0.002	-
7) Gr. Beef #7		Pork	0.066	0.003	-
8) Gr. Beef #8		Pork	0.850	0.013	+
9) Gr. Beef #9		Pork	0.050	0.001	-
10) Gr. Beef #10		Pork	0.461	0.005	+
11)					
12)					
13)					
14)					
15)					
16)					
17)					
18)					
19)					
20)					

VALID

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.

SPECIES TEST KITS AVAILABLE

MELISA-TEK® COOKED MEAT SPECIES KITS	**CAT NO:**
***MELISA-TEK®* RUMINANT** (Bovine/Ovine) KIT	SE110017
***MELISA-TEK®* PORK** KIT	SE110018

ELISA-TEK® COOKED MEAT SPECIES KITS	**CAT NO:**
COOKED MEAT **3 Species** (Beef,Pork,Poultry)	SE110008
COOKED MEAT **4 Species** (Beef,Pork,Poultry,Sheep)	SE110009
COOKED MEAT **BEEF** KIT	SE110001
COOKED MEAT **PORK** KIT	SE110002
COOKED MEAT **POULTRY** KIT	SE110003
COOKED MEAT **SHEEP** KIT	SE110004
COOKED MEAT **HORSE** KIT	SE110005
COOKED MEAT **DEER** KIT	SE110006

ELISA-TEK®RAW MEAT SPECIES KITS	**CAT NO:**
RAW MEAT **3 SPECIES** KIT(Beef,Pork,Poultry)	SE110015
RAW MEAT **4 SPECIES** KIT(Beef,Pork,Poultry,Sheep)	SE110016
RAW MEAT **BEEF** KIT	SE110010
RAW MEAT **PORK** KIT	SE110011
RAW MEAT **POULTRY** KIT (Detects all poultry)	SE110012
RAW MEAT **SHEEP** KIT	SE110013
RAW MEAT **HORSE** KIT	SE110014

SIGMA-ALDRICH®

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800)521-8956 Fax: (800)325-5052
Sigma-Aldrich.com

ELISA Technologies, Inc.
2501 NW 66th Court
Gainesville, Florida 32653 USA
Tel: (352) 337-3929
Fax: (352) 337-3928
E-Mail: info@elisa-tek.com
Web: www.elisa-tek.com