ELISA-TEK®

COOKED MEAT SPECIATION KITS

For the Identification of Animal Species Content in Cooked and Canned Meat & Poultry Products by Enzyme Linked ImmunoSorbent Assay (ELISA)

INSTRUCTIONS FOR USE

SIGMA-ALDRICH®

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INTRODUCTION

Preventing adulteration of meat foods with less desirable or objectionable meat species is important for economic, regulatory, health and ethnic/religious reasons. The identification of meat species is performed in many countries to assure consumers that the meat and poultry they purchase is unadulterated and properly labeled.

The **Cooked Meat Species ELISA**, developed and utilized by the USDA, is based on antibodies raised to heat-resistant, species-specific, muscle-related glycoproteins and employs the principle of the **Enzyme-Linked ImmunoSorbent Assay (ELISA)**. ***ELISA-TEK®* COOKED MEAT SPECIATION KITS** are sensitive and specific tests designed to determine the species content in cooked meat and poultry products. The ***ELISA-TEK®* COOKED MEAT SPECIATION KITS** have been formatted and refined for ease of use and will meet or exceed **USDA-FSIS protocol standards for the Cooked Meat Species ELISA**.

This kit may also be used with the ***ELISA-TEK*** protocol that allows for flexibility in sample matrix and slightly higher sensitivity.

NOTE: This manual contains a direct adaptation of "Identification of Animal Species in Cooked and Canned Meat and Poultry Products" by Ronald G. Berger[1] as found in the United States Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook[2]. Following the USDA Procedures given in this manual and the USDA-FSIS MLG[2] will enable the user of the ELISA-TEK® MICROWELL Kits for Cooked Meat Speciation to produce assay results according to official USDA-FSIS protocols.

PRINCIPLE OF THE TEST

***ELISA-TEK®* COOKED MEAT SPECIATION KITS** will identify the species of animal tissue used as ingredients in cooked meat foods by employing a simple extraction of samples and an amplified, double sandwich type **E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay.

With this type of ELISA, capture antibody is bound to the plastic of the microplate wells. The sample extract is added to the wells and tissue antigens of the species of interest are bound by the capture antibody in the wells. After washing to remove unbound material, a secondary antibody with the same species specificity as the capture antibody is added. This biotinylated antibody binds to any antigens captured on the plate; otherwise, the unbound biotinylated antibody is removed by washing. Streptavidin-horseradish peroxidase conjugate is then added to the wells and binds to the immobilized biotinylated antibodies. Unbound conjugate is removed by washing. A substrate (ABTS) for the enzyme is then added to the wells. If species antigens were present in the test sample, a green color will develop as a result of the action of the bound enzyme on the substrate. The color development may be read photometrically by using a microplate reader.

NOTE: The Cooked Meat Species ELISA is intended for use as a qualitative test only. The color development is proportional to the original amount of species antigen in the extract, but one should not attempt to quantify the amount of species tissue in a sample based on this assay. Variations in sample content (e.g. % lean tissue, % moisture, % fat, etc.) and variations in sample treatment (e.g. cooking times, temperatures, etc.) will affect the amount of detectable antigen in the extract.

KIT CONTENTS

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 held in a plastic frame and packed in a foil laminate pouch with a desiccant. Each strip has eight microwells (96 test wells total). Each microwell has been coated with a calibrated amount of species-specific antibody, freeze-dried, and labeled according to its specificity.

B. **THREE or MORE** vials of **SPECIES CONTROLS** containing 1.5 ml each of control in buffered solution with 0.04% sodium azide as a preservative. Each serves as a positive control in the appropriate test, and can also be used as a negative control in any other Cooked Meat Species test.

C. **THREE or MORE** vials of **BIOTINYLATED ANTI-SPECIES ANTIBODY** containing 1.0 ml of a calibrated, buffered, antibody solution containing carrier serum, a wetting agent, and 0.04% sodium azide as a preservative.

D. **ONE** vial of **STREPTAVIDIN-PEROXIDASE CONJUGATE** containing 6.0 ml of conjugate in a buffered solution.

E. **ONE** vial of **ABTS CONCENTRATE** containing 1.1 ml of ABTS (1.5% 2,2' azino-di-(3-ethyl-benzthiazoline sulphonic acid) in water.

F. **ONE** vial of **PEROXIDE CITRATE BUFFER** containing 12.0 ml of citrate buffer solution with hydrogen peroxide.

G. **ONE** vial of **STOP SOLUTION** containing 6.0 ml (1.5% w/v sodium fluoride in water).

H. **ONE** bottle of **WASH SOLUTION CONCENTRATE** containing 100 ml of a ten-fold (10X) concentrate of Tris buffered saline with a wetting agent and 0.02% thimerosal as a preservative.

I. **ONE INSTRUCTION MANUAL**.

J. **TWO BLANK WORKSHEET/RESULT FORMS.**

KIT STORAGE INSTRUCTIONS

***ELISA-TEK®* COOKED 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 re-dried by placing in a 100°C oven (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).

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.

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 should be treated by washing/irrigation. It is also important to be aware of the allergic, toxic or infectious potential of analytical samples.

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents: Purified water, Sodium Chloride

Equipment: Miscellaneous laboratory plastic and/or glassware, equipment for sample handling/extraction and containers suitable for meat extracts; Whatman no. 4 or similar filter paper for clarifying sample extract; Precision micropipette and tips capable of delivering 25, 50 and 100 microliter volumes; Wash bottle for washing microwells between reagent additions.
NOTE: *USDA Procedures specify a dual wavelength microwell plate reader, fitted with 414nm (405-420nm acceptable) and 492nm (485-500nm acceptable) filters.

Optional: Domestic blender, mincer or stomacher and suitable sample preparation bags; Centrifuge and appropriate centrifuge tubes; Precision repeating dispenser, and tips capable of delivering 25, 50 and 100 microliter volumes; Precision multichannel pipetter and tips capable of delivering 25, 50 and 100 microliter volumes; Microwell washer.

PROCEDURAL NOTES AND PRECAUTIONS

1. Review the complete instructions before performing the ***ELISA-TEK®*** Cooked Meat Species ELISA.

2. The ***ELISA-TEK® Cooked 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 and 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 room 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 not to 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 cooked. If uncooked samples are to be tested, or if it is suspected that the sample is not fully cooked, it is advisable to heat the extract (meat/saline mixture) in a water bath at 95-100°C for 15 minutes prior to mixing and filtration/centrifugation.

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.

SAMPLE PREPARATION AND EXTRACTION

NOTE: 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.

NOTE: If using the ELISA-TEK protocol, it is recommended that the end user prepare a negative control, which is matrix-matched to the unknown sample(s). This negative control is prepared in the same way as the unknown sample(s).

1. Prepare a product for testing by dicing or finely chopping.

2. Weigh 5 grams of the diced sample in a 6 oz. stomacher or whirl-pak bag.

3. Add 10 milliliters normal saline (0.9% [0.15M] Sodium Chloride).

4. Place bag and contents into a stomacher for 60 seconds. Alternately, the mixture may be kneaded or manually homogenized.

5. Remove from the stomacher and leave undisturbed for 1 hour at room temperature.

   NOTE: All samples to be tested must be COOKED. If uncooked samples are to be tested, or if it is suspected that the sample is not fully cooked, it is advisable to heat the extract (meat/saline mix) in a water bath at 95-100°C for 15 minutes prior to mixing and centrifugation/filtration.

   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, as described in step 6.

6. Filter a portion of the liquid through a Whatman 4 (or similar) filter paper. Alternatively, you may pour off some of the liquid into a centrifuge tube and centrifuge at 10,000 X *g* for 10 minutes.

7. The clear supernatant tissue extract is used in the ELISA.

   NOTE: If the sample has a high fat content, the clear supernatant above the settled meat layer may be beneath a layer of fat. Avoid transferring the fat. It may be appropriate to carefully remove a portion of the aqueous solution using a clean (e.g. Pasteur-type) pipette into a second, clean container prior to analysis.

EXTRACTED SAMPLE STORAGE:

Sample Extracts may be stored at 2-8°C for up to 36 hours. If prolonged storage is required, the extracts must be kept frozen. They will remain stable for several months when stored at -20°C.

PREPARING A PLATE PLAN

ELISA-TEK® *Cooked Meat Speciation Kits* 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, the species to be tested and the number of replicates desired**. IT IS IMPORTANT** to prepare a test layout showing the wells you will use for controls and samples in the following protocol. Ideally each assay will include replicates of a 100% POSITIVE TISSUE CONTROL, a 1% POSITIVE TISSUE CONTROL, and one or more NEGATIVE TISSUE CONTROLS. For the POSITIVE CONTROL use the appropriate kit control (e.g., pork in the pork test) or a lean tissue extract of the species of interest. For the 1% POSITIVE TISSUE CONTROL, use a 1% dilution (e.g., 10 µl control + 990 µl normal saline) of the appropriate species positive control or of a lean tissue extract of the appropriate species. For the NEGATIVE CONTROLS, use one or more appropriate kit controls (e.g., beef in the pork test) and the matrix-matched negative control provided and prepared by the end user. Decide on the number of replicates of each control and sample extract you will run. For screening samples, single or duplicate microwells for each control and sample extract may be adequate.

NOTE: USDA-FSIS protocols require use of quadruplicate microwells for each control and for sample extracts that are potental violations

PREPARATION OF KIT MATERIALS

A. ANTIBODY SENSITIZED 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. CONTROLS: Ready to use as 100% controls; dilute appropriate control to prepare1% positive control (e.g., 10 µl of 100% control plus 990 µl of normal saline)

C. ANTI-SPECIES BIOTINYLATES: Ready to use

D. STREPTAVIDIN PEROXIDASE CONJUGATE: Ready to use

E/F. ABTS CONCENTRATE and PEROXIDE CITRATE BUFFER: Mix contents of each separate vial by inversion. DO NOT SHAKE. ABTS is supplied as a 25-fold concentrate and must be diluted in PEROXIDE CITRATE BUFFER to prepare WORKING ABTS SOLUTION.

- For 96 test wells add 0.5 mL (500 µL) of ABTS CONCENTRATE to the 12.0 mL of PEROXIDE CITRATE BUFFER. Stopper the vial and mix well by gentle, repeated inversion or swirling.
- For any other number of test wells, dilute ABTS CONCENTRATE in a 1:25 ratio with PEROXIDE CITRATE BUFFER (e.g. for each strip of 8 test wells, pipette 0.6 mL Peroxide Citrate Buffer into a clean container and add 0.025 mL (25 µL) ABTS Concentrate. Mix well by swirling gently.

NOTE: Dilutions of ABTS CONCENTRATE should be made fresh just prior to use (e.g., during the avidin peroxidase conjugate incubation*).*

G. STOP SOLUTION: Ready to use

H. WASH SOLUTION CONCENTRATE: WASH SOLUTION CONCENTRATE is supplied as 100 mL of a 10-fold concentrate and requires dilution in purified water to prepare WORKING WASH SOLUTION (100 mL concentrate plus 900 mL diH2O)

PROCEDURE SUMMARY FLOW CHART

PROCEDURE	VOLUME	TIME	DESCRIPTION
Addition	100µL	-	Pipette **NORMAL SALINE**, **CONTROL** & **SAMPLE EXTRACTS** into respective test wells
Incubate	-	1 hr	Incubate at room temperature
Wash	-	-	Wash each well 3 times using WORKING WASH SOLUTION
Addition	25µL	-	Pipette **ANTI-SPECIES BIOTINYLATE** into all test wells
Incubate	-	1 hr	Incubate at room temperature
Wash	-	-	Wash each well 3 times using WORKING WASH SOLUTION
Addition	25µL	-	Pipette **AVIDIN PEROXIDASE CONJUGATE** into all test wells
Incubate	-	30 min	Incubate at room temperature
Wash	-	-	Wash each well 6 times with WORKING WASH SOLUTION
Addition	50µL	-	Pipette **WORKING SUBSTRATE SOLUTION** into all test wells
Incubate	-	30 min	***ELISA-TEK PROTOCOL***: Incubate 30 minutes at room temperature
			OR
		5-30 min	***USDA PROTOCOL***: Incubate at room temperature until 414-492 O.D. of positive control is between 0.450-0.500.
Addition	50µL	-	Pipette **STOP SOLUTION** into all test wells and mix by gently rotating the microplate
Results	-	-	Measure absorbance of each well at 414-492nm using a microplate reader and perform data analysis

DETAILED ENZYME IMMUNOASSAY PROCEDURE

1. Remove reagents from the box and allow them to reach room temperature before starting the test.

2. Prepare the sample extracts (pg. 7), matrix-matched negative control sample (if using) and other kit materials (pg. 8).

3. Remove your microwell assembly from its pouch and, referring to your plate plan, (see page 8) 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. Using a precision micropipette, place 100µL of normal saline in each of the wells selected as blanks.

NOTE : Be sure to use a clean pipette tip for each control and sample to be tested .

5. Place 100µL of each NEGATIVE CONTROL in each of the wells selected.

6. Place 100µL of 1% POSITIVE CONTROL in each of the wells selected.

7. Place 100µL of 100% POSITIVE CONTROL in each of the wells selected.

8. Place 100µL of each SAMPLE EXTRACT in each of the wells selected. Avoid transferring any fat from sample preparations to the wells.

9. Mix the plate gently by hand; cover. Allow to stand at room temperature for 1 hour.

10. At the end of the incubation period, empty the wells by flicking into the sink. Then carefully fill all wells with Working Wash Solution using a reagent wash bottle. Repeat the emptying and filling of each well twice more and then emptying into the sink. Invert the aspirated/emptied 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.

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 the plate 3 times. Invert the aspirated/emptied plate and rap sharply several times onto a soft paper towel placed on the lab bench.

DETAILED ENZYME IMMUNOASSAY PROCEDURE Cont'd

11. Add 25µL of ANTI-SPECIES BIOTINYLATE to each microwell of the relevant (same species) ANTIBODY SENSITIZED STRIP(S). Work from the top to the bottom of each strip. Place a fresh tip in the micropipetter and repeat the biotinylate additions as necessary for each species being run.

NOTE: Observe that the bottom of each well is covered with liquid. If not, gently tap the edge of the plate until this is accomplished. Avoid getting any antibody on the sides of the wells.

12. Cover the plate and leave at room temperature for 1 hour.

13. Repeat wash step 10.

14. Add 25µL of PEROXIDASE CONJUGATE to each well. Again, observe that the bottom of each well is covered with liquid and that no conjugate sticks to the sides of the wells.

15. Cover the plate and leave at room temperature for 30 minutes.

16. Repeat wash step 10, except wash six times instead of three.

17. Add 50µL of the WORKING ABTS SOLUTION to each microwell (see also preparation of WORKING ABTS SOLUTION as described on page 8, E/F).

18. ***ELISA-TEK PROTOCOL***: Cover the plate and incubate at room temperature for 30 minutes

    OR

    USDA PROTOCOL: Observe the microwells containing the 100% POSITIVE CONTROL for visual color change. When color change is observed, place the plate on the reader carriage. Read and obtain 414-492nm O.D. values for the wells. Continue to read until 100% POSITIVE CONTROL O.D. values read in a range of 0.450 to 0.500 (refer to USDA-FSIS MLG[2] for more detailed instructions).

19. Quickly add 50µL of STOP SOLUTION to each microwell.

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

21. Read the completed assay with the aid of a microplate reader at O.D. 414-492.

DETERMINATION OF TEST RESULTS

Program your microplate reader to read absorbance at 414nm (405-420nm acceptable) and then read and subtract the absorbance at 492nm (485-500nm acceptable) from the 414nm reading.

1. Place the microplate on the reader carriage and blank the instrument on the selected blank (normal saline) wells. Alternately, read all wells as raw O.D. values and manually subtract the average value of the normal saline wells from the average O.D.'s of the controls and samples.

2. Read and obtain a printed copy of the absorbance values for each well.

3. Determine the blank-subtracted mean absorbance value and standard deviation of each of the POSITIVE CONTROL, 1% POSITIVE TISSUE CONTROL, NEGATIVE CONTROLS, and SAMPLE wells.

4. **In order for the assay to be valid according to the *ELISA-TEK* PROTOCOL the following conditions must be met:** the blank-subtracted mean O.D. of the 1% positive control of the appropriate species must be greater than 0.250, the blank subtracted mean O.D.s of each of the negative controls must be less than 0.150, and the standard deviation of the replicates must be less than 0.060. If these conditions are met then the test is valid. Otherwise the test is invalid, and should be repeated.

5. **In order for the assay to be valid according to USDA PROTOCOL the following conditions must be met:** the blank-subtracted mean O.D. of the 100% POSITIVE CONTROL of the appropriate species must be greater than 0.600, the blank-subtracted mean of the negative controls must be less than 0.060, and the standard deviation of the replicates must not be more than 0.060. If these conditions are met then the test is valid. Otherwise the test is invalid, and should be repeated.

6. **If the assay is determined to be valid by the above parameters, the unknown samples may be classified as follows:**

   ***ELISA-TEK* PROTOCOL**:

   There are two criteria which can be used to determine the status of unknown samples by this protocol. It is up to the end user to decide which criterion is valid for their samples. The kits have been validated to meet criterion 1 in our laboratory.

   CRITERION 1 – Any sample whose mean blank-subtracted O.D. value, minus three standard deviations, is greater than 0.250 will be considered positive; all other samples are negative**.**

   CRITERION 2 – Any sample whose mean blank-subtracted O.D. value minus three standard deviations is higher than 0.100 **and** higher than the mean O.D. value of the highest negative control (either kit-supplied or the matrix-matched control prepared by the end user) plus three standard deviations is positive; all other samples are negative. It is recommended that the end user extract and test an appropriate matrix-matched negative control in order to use this criterion.

   USDA PROTOCOL

   Any sample whose mean blank-subtracted O.D. value, minus three standard deviations, is greater than 0.250 will be considered positive; all other samples are negative**.** Report Below Regulatory Action Levels (BRAL) for meat species or sample types that are known to not cross-react and that are positive according to *ELISA-TEK* Protocol Criterion 2 (above).

PERFORMANCE CHARACTERISTICS

ELISA-TEK® COOKED MEAT SPECIATION KITS, when used as directed, will produce results that meet USDA criteria, and will identify cooked meat and poultry samples containing the species of interest at levels of approximately 1% or greater.

In our laboratory, uncooked, lean meat samples prepared as directed 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).

ELISA-TEK® COOKED MEAT SPECIATION KITS are designed to give optimal performance at room temperatures between 20°C and 25°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 degree of color of the 100% POSITIVE CONTROL (O.D. = 0.600 to 0.900 @ 414-492nm).

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 medium green coloration.

Significant visible color (O.D. @ 414-492nm >0.150) in any of the blank or negative control wells may indicate contamination of the WORKING ABTS 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 for cross-reaction and has been found to produce negative responses to the heterologous species samples.

It should be noted that samples of the feathered species (e.g., chicken, turkey, game hen, goose, duck etc.) all produce positive responses in the COOKED POULTRY assay. Similarly, a bison sample will produce a positive result in the COOKED BEEF assay, and a goat sample will produce a positive response in the COOKED SHEEP assay. Further details of responses to other and closely related species is available on request.

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 cooked 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 the discretion of ELISA Technologies, Inc.

SAMPLE PLATE PLAN FOR SINGLE-SPECIES TEST:

ASSAY: Cooked Pork **OPERATOR:** D.M. **DATE:** 12-10-10

CONTROL or SAMPLE ID
1) Pork Pos. Cont.
2) Bf. Neg. Cont.
3) 97-1: Pepperoni
4) 97-2: Cocktail
5) 97-3: Bologna
6) 97-4: Jalapeño
7) 97-5: Beef
8) 97-6: Frank
9) 97-7: Polish
10) 97-8: Kielb.
11)
12)
13)
14)
15)
16)
17)
18)
19)
20)
21)
22)
23)
24)
25)

PLATE LAYOUT PLAN: (See also page 8)

◄ 1) Identify & Record Controls and Samples

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

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

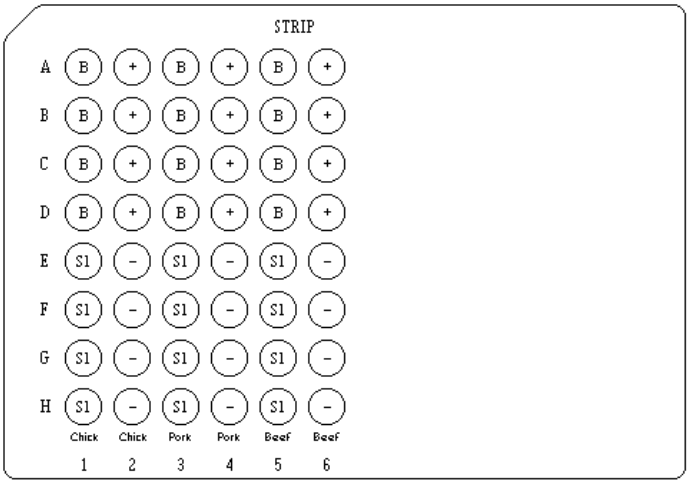
▲ 3) Plot Location of Controls and Samples:

PROCEDURE RECORD: (See also pages 10 - 11)

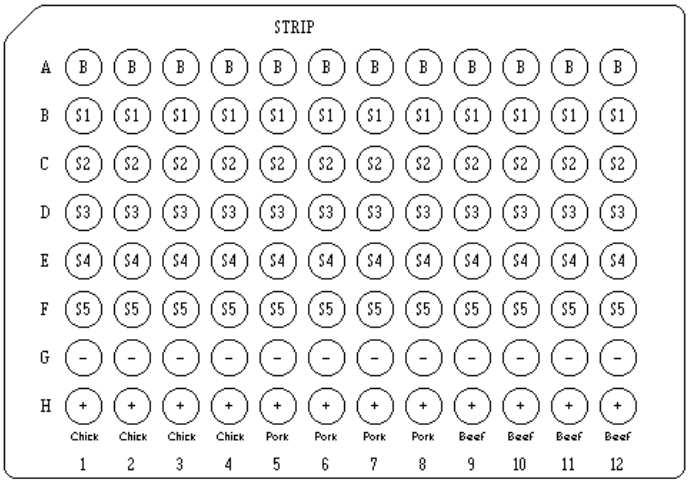
▼ 4) Record Procedure Times and Temps:

Step #	Procedure	Volume to Add	Incubate For	Time Added	Temp. (Room)
4 - 8	Add Controls and Samples	100 µL	60 min.	11:54	23.0
11	Add Anti-Species Conjugates	25 µL	60 min.	1256	23.0
14	Add Avidin-Peroxidase	25 µL	30 min.	1:57	23.5
17	Add Working ABTS Solution	50 µL	5-30 min.	2:31	23.5
19	Add Stop Solution	50 µL		Read & Record	Page 12

EXAMPLE 6-STRIP, 3-SPECIES PLATE LAYOUT



EXAMPLE 12-STRIP, 3-SPECIES PLATE LAYOUT



B= NORMAL SALINE BLANK +=100% POSITIVE CONTROL - = 100% NEGATIVE CONTROL
S1-S5 = SAMPLE EXTRACTS

SAMPLE RESULTS FORM

ASSAY: Cooked Pork **DATE:** 12-10-10

CONTROL SPECIES	CONTROL ID or LOT #	SPECIES TESTED	MEAN ABSORBANCE	STANDARD DEVIATION	VALID or INVALID NEG <0.060 Pos ➾ 0.600
NS	71210A	Pork	0.026	0.002	VALID
1% Pork	71210B	Pork	0.405	0.015	VALID
Beef	70808	Pork	0.028	0.001	VALID
Pork	71105	Pork	0.844	0.042	VALID
SAMPLE IDENTIFICATION		SPECIES TESTED	MEAN ABSORBANCE	STANDARD DEVIATION	SAMPLE RESULT Pos ➾ 0.250
1) 97-1: Beef Pepperoni		Pork	0.859	0.030	POSITIVE
2) 97-2: Cocktail Frank		Pork	0.029	0.002	NEGATIVE
3) 97-3: Beef Bologna		Pork	0.040	0.002	NEGATIVE
4) 97-4: Jalapeño Frank		Pork	0.039	0.002	NEGATIVE
5) 97-5: Beef Frank		Pork	0.103	0.005	NEGATIVE
6) 97-6: Frank		Pork	0.034	0.002	NEGATIVE
7) 97-7 Polish Sausage		Pork	0.369	0.016	POSITIVE
8) 97-8: Beef Kielbasa		Pork	0.035	0.001	NEGATIVE
9)					
10)					
11)					
12)					
13)					
14)					
15)					
16)					
17)					
18)					
19)					
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REFERENCES

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SPECIES TEST KITS AVAILABLE

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***MELISA-TEK®* RUMINANT** (Bovine/Ovine) KIT	SE110017
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ELISA-TEK® COOKED MEAT SPECIES KITS	**CAT NO:**
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COOKED MEAT **4 Species** (Beef,Pork,Poultry,Sheep)	SE110009
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COOKED MEAT **PORK** KIT	SE110002
COOKED MEAT **POULTRY** KIT	SE110003
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COOKED MEAT **HORSE** KIT	SE110005
COOKED MEAT **DEER** KIT	SE110006

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