

MELISA-TEK®

SPECIATION KITS FOR MEAT & BONE MEALS
AND ANIMAL FEEDS



For the Detection of Animal Species Content
Of Meat & Bone Meals and Animal Feeds by
Monoclonal Enzyme Linked ImmunoSorbent Assay

INSTRUCTIONS FOR USE

MELISA-TEK® RUMINANT KIT - Cat. No. 510311
MELISA-TEK® PORK KIT - Cat. No. 510321

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CONTENTS

REVISION 110303-v2

Page Number	
2	TABLE OF CONTENTS
3	GENERAL DESCRIPTION
3	TEST PRINCIPLE
3	IMPORTANCE OF TROPONIN-I DETECTION
3	SAFETY INSTRUCTIONS
3	STORAGE AND STABILITY
4	MATERIALS PROVIDED
4	MATERIALS NOT PROVIDED
5	PREPARING A PLATE PLAN
6	KIT COMPONENT PREPARATION
6	PREPARE SAMPLES TO BE TESTED
6	PREPARE THE TEST CONTROLS
7	ASSAY PROCEDURE
8	RESULTS WORKSHEET
8	PERFORMANCE CHARACTERISTICS
9	KIT SPECIFICITY
9	KIT LIMITATIONS
9	DISCLAIMER
10	REVISION SUMMARY
11	SPECIES KITS LISTING

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MELISA-TEK[®] SPECIES ASSAY KIT INSTRUCTIONS

General Description:

The ELISA Technologies **MELISA-TEK[®]** Species assay kit is an immunoassay for the detection of species specific troponin-I. Troponin-I is a heat stable, muscle specific protein and, as such, this kit is intended to detect muscle tissue in extracts made from cooked meat and feed products such as meat meals and meat and bone meals.

Test Principle:

This test is a sandwich ELISA that allows for detection of muscle tissue in extracts made from cooked meat or feed products (e.g. meat meals and meat and bone meals). It is based on the recognition of troponin-I, a heat-stable muscle-specific protein, by specific monoclonal antibodies. One troponin-I-specific monoclonal antibody is immobilized to the wells of the test strips, which captures troponin-I present in the samples or controls. After a wash step, a second troponin-I-specific monoclonal antibody, which has been biotinylated, is allowed to bind to the troponin-I present in the well. After a second wash step, a streptavidin-horseradish peroxidase (SA-HRP) conjugate is added which binds to the biotinylated secondary antibody, and any unbound SA-HRP is washed away. The TMB substrate is added, which reacts with the HRP of the conjugate, causing a color change in proportion to the level of troponin-I originally bound to the well. Finally, a stop solution is added after a specific time and color development is evaluated using an ELISA plate reader.

Importance of Troponin-I Detection:

Determination of the presence of animal protein content in feed products such as meat and bone meal is of interest due to concern over Bovine Spongiform Encephalopathy (BSE) and other diseases, and for religious reasons. Regulations passed by Great Britain, the EU, Japan and the United States require that ruminant animal feeds contain no ruminant animal carcass. Detection of thermo-stable troponin-I allows for the detection of muscle tissue in processed and heat-treated feed products such as meat and bone meal, while not detecting exempted materials such as ruminant milk, blood or gelatin.

Safety Instructions:

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.

Storage and Stability:

The **MELISA-TEK[®]** Kits should be stored at 4-8°C. **DO NOT FREEZE**. All components of the kit should be allowed to reach room temperature (20-25°C) before use, and any unused reagents should be returned promptly to the refrigerator. Unused plate strips must be stored in their sealed bag with desiccant packets. Reagents may be used until the expiration date on the box.

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MATERIALS PROVIDED

- 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 and labeled according to its specificity.
- B. **THREE** vials of **BIOTINYLATED ANTI-SPECIES ANTIBODY** containing 2.0 ml of a calibrated, buffered, antibody solution containing carrier serum, a wetting agent, and 0.04% sodium azide as a preservative.
- C. **THREE** vials of **SPECIES MUSCLE TISSUE CONTROL (10%)** containing **4 ml** each of a species muscle tissue extract diluted in buffered solution with 0.04% sodium azide as a preservative. Each serves as a positive control in the appropriate test and as a negative control and as a diluent for preparing diluted controls in heterologous tests.
- D. **ONE** vial of **AVIDIN-PEROXIDASE CONJUGATE** containing 6.0 ml of conjugate in a buffered stabilizer.
- E. **ONE** vial of **TMB** containing 6.0 ml buffered and stabilized TMB (Tetramethylbenzidine).
- F. **ONE** vial of **STOP SOLUTION** containing 6.0 ml (25% w/v Phosphoric acid in water).
- G. **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.
- H. **THREE Packets of EXTRACTION BUFFER SALTS**, each containing a mixture of phosphate saline buffer salts and EDTA sufficient to make 1 liter of EXTRACTION SOLUTION.
- I. **ONE INSTRUCTION MANUAL.**
- J. **TWO BLANK WORKSHEET/RESULT FORMS.**

Materials Not Provided:

1. Purified water.
2. Precision micropipettors and tips with a range of 20-1000 ul.
3. 250 ml flasks for sample extracts.
4. Domestic blender or mortar and pestle to reduce size of compressed feeds.
5. Stomacher for sample preparation.
6. Plastic or glass vials for preparing control dilutions.
7. One-liter containers for preparing Extraction Solution and Wash Solution.
8. Water bath for heating sample extracts to 100°C.
9. Whatman No.4 or similar filter paper for clarifying sample extracts.
10. ELISA plate or strip washer.
11. ELISA plate reader with a 450nm filter.

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PREPARING A PLATE PLAN

The **MELISA-TEK®** Kit 96 microwell unit 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 following the protocol you have chosen. This layout plan will be used to determine the number of strips of each species you will need to use, the locations and volumes needed of samples, controls, and species specific reagents during the procedure, and to locate and identify the data/result for each control and sample. Blank plate plans are included in the kit. An example of a possible format is shown below.

For Screening: Duplicate wells of each control and sample extract are recommended.
 For Confirmatory Testing: Triplicate or Quadruplicate wells of each control and sample extract are recommended

- 1) Determine the number and type of controls you wish to use for each species you are testing for and designate which wells they will occupy on the plate.
- 2) Determine the number of replicates of sample extracts you will be testing and designate which wells they will occupy on the plate.
- 3) Locate one of the enclosed worksheet templates showing the 96 well layout. Mark the location of the wells selected for each control and sample extract on the Plate plan worksheet.
- 4) Sample Plate Plan:

STRIP	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B									
B	LPC	LPC	LPC									
C	HPC	HPC	HPC									
D	NEG	NEG	NEG									
E	1	1	1									
F	2	2	2									
G	3	3	3									
H	4	4	4									
SPECIES	Rum	Rum	Rum	Rum	Rum	Rum	Rum	Rum	Rum	Rum	Rum	Rum

Blank = Extraction Solution Only
 LPC = Low Positive Control (0.05%)
 HPC = High Positive Control (1%)
 NEG= Negative Control (alternate species control)
 S# = Sample Number

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TEST PREPARATION

1. Kit Component Preparation

- a. Allow all kit components to reach room temperature.
- b. Prepare Extraction Solution by mixing contents of one Extraction Solution packet in 1 liter of purified water. Mix until contents are completely dissolved.
- c. Prepare Wash Solution by mixing one bottle of Wash Solution concentrate (100ml) with 900 ml of purified water. Mix by inverting gently several times.

2. Prepare samples to be tested:

- a. For compressed feed samples, reduce the particle size using a domestic blender or mortar and pestle.
- b. Weigh 5.0 grams of the sample into a 250 ml flask.
- c. Add 50 ml of Extraction Solution and allow the sample to pre-swell for 30 minutes at room temperature
- d. Cover flask with foil and heat in a 95-100°C water bath for 15 minutes.
- e. Remove from the water bath and allow to cool (no more than 30 minutes).
- f. Filter the extract liquid through Whatman no.4 filter paper. Alternatively, pour the sample into a centrifuge tube and centrifuge at 10,000 x G for 10 minutes.
- g. The extract may be clear or cloudy. It requires no further dilution for use in the assay.

NOTE: If using the **MELISA-TEK®** High Sensitivity Sample Extraction Kit, Cat. No, 510391, the sample preparation steps shown above will be replaced by the procedures given in the instructions included with that kit.

3. Prepare the test controls:

- a. The positive controls in the kit are 10% dilutions of skeletal muscle extracts. Use the species control that matches the species you are trying to detect in your samples. The recommended controls for use in the **MELISA-TEK®** assay are a 1% High Positive Control and a 0.05% Low Positive Control, which are prepared as follows:
 - i. To prepare the 1% High Positive Controls, add 100 ul of the appropriate supplied 10% positive control to 900 ul of Extraction Solution. Mix gently by pipetting.
 - ii. To prepare the 0.05% Low Positive Control, add 50 ul of the 1% High Positive Control (prepared above) to 950 ul of the 10% Negative Control. Mix gently by pipetting.
- b. Each species control included with the kit is a 10% dilution of a species tissue extract. When used as a negative control they do not need to be diluted before being used in the assay,
- c. *Diluted Positive controls must be used the day they are prepared and then discarded.*

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ASSAY PROCEDURE

1. Pipette 100ul of extraction solution (blank), controls and samples into the wells of the test strips according to the plate plan. We recommend using triplicate wells for each.
2. Incubate 20 minutes at room temperature.
3. Wash the wells 3 times using the prepared Wash Solution and a plate or strip washer.
4. Pipette 50ul of Biotinylated Secondary Antibody into each well.
5. Incubate 20 minutes at room temperature.
6. Wash the wells 3 times.
7. Pipette 50ul of Avidin-Peroxidase Solution into each well.
8. Incubate 20 minutes at room temperature
9. Wash the wells 6 times.
10. Pipette 50 ul of TMB Substrate into each well.
11. Incubate 20 minutes at room temperature – DO NOT WASH.
12. Pipette 50 ul of Stop Solution into each well.
13. Read the plate on a 96-well plate reader with a 450nm filter. Read the plate within 10 minutes of adding stop solution

Assay and Sample Evaluation:

1. Take the average of the triplicate values for the blank and each sample and control. This can be done on the worksheet provided.
2. Subtract the average blank OD from the average OD of each sample and control, and record these blank-subtracted values on the worksheet.
3. The assay is considered **VALID** if:
 - a. The blank-subtracted OD of the 1% High Positive Control is greater than 1.000
AND
 - b. The blank-subtracted OD of the 0.05% Low Positive Control is greater than 0.100
AND
 - c. The blank-subtracted OD of the 10% Negative Control is less than 0.100
AND
 - d. The standard deviation of the 0.05% Low Positive Control replicates is no more than 0.100.
4. If these conditions are not met, the test is **INVALID** and should be repeated.
5. If the assay is valid, then the samples may be classified as positive or negative.
6. Test Sample Classification:
 - a. Test samples may be classified as **POSITIVE** if the blank-subtracted average OD is **GREATER** than 0.100 and the controls indicate a valid test.
 - b. Test samples are considered **NEGATIVE** if the blank-subtracted average OD is **LESS** than 0.100 and the controls indicate a valid test.

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SAMPLE RESULTS WORKSHEET



MELISA-TEK[®] RESULTS FORM

ASSAY: MELISA-TEK SPECIES: Ruminant ASSAY DATE: March 1, 2007

KIT LOT: M2M70228-30 OPERATOR: ESP

CONTROL RESULTS:

CONTROLS	ABSORBANCE @450 nm				STD DEV	Average OD Minus Blank OD	ASSAY VALIDITY	
	OD 1	OD 2	OD 3	Average OD				
Extraction Soln (Blank)	0.082	0.076	0.079	0.079	0.002	0.000	Control OD	Pass/Fail
Negative Species LSMT (10%)	0.079	0.085	0.082	0.082	0.002	0.003	NC ≤ 0.100	Pass
							NC ≤ 0.100	
Positive Species LSMT (0.05%)	0.985	0.931	0.958	0.958	0.022	0.879	LP ≥ 0.100	Pass
							LP ≥ 0.100	
Positive Species LSMT (1.0%)	3.991	4.000	3.958	3.983	0.018	3.904	HP ≥ 1.000	Pass
							HP ≥ 1.000	

SAMPLE RESULTS:

SAMPLES	ABSORBANCE @450 nm				Extraction Blank OD	Average OD Minus Blank OD	SAMPLE RESULT ABS OD ≤ 0.100 = NEG ABS OD > 0.100 = POS
	OD 1	OD 2	OD 3	Average OD			
1) <u>E770308-1</u>	0.094	0.099	0.097	0.097	0.079	0.018	NEGATIVE
2) <u>E770308-2</u>	0.114	0.121	0.129	0.121	0.079	0.042	NEGATIVE
3) <u>E770308-3</u>	0.846	0.878	0.867	0.864	0.079	0.785	POSITIVE
4) _____							
5) _____							

Performance Characteristics:

The **MELISA-TEK[®] SPECIES KITS**, when used as directed, will identify the presence of thermo-stable muscle tissue protein (TSMP) in meat & bone meals and animal feed samples containing muscle tissue at levels of approximately 0.05% or greater. (Approximately 1% EU MBM [AMI & AMII] or 5% EU MBM in Feeds).

In house testing indicates the following detection limits:

1. Muscle Tissue: Lean muscle tissue prepared at up to 138°C + 4 bars pressure X 20 minutes in a closed container is detected at a 1:2000 dilution of a 1:10 extract prepared as described on page 6, equivalent to a concentration of 0.005% lean muscle tissue in a sample.
2. Meat and Bone Meal: International Reference Materials and Measurements, (IRMM) meat and bone meals processed at 133°C + 3 bars pressure X 20- minutes composed of 50% beef/50% pork is detected at a 1:100 dilution of an extract prepared as described on page 6, equivalent to a concentration of 0.5% meat and bone meal in a sample.
3. Animal Feed: Species TSMP antigens are detected in animal feeds containing 5% IRMM Meat and bone meal (50% beef/50% pork) when extracted as described on page 6, equivalent to a concentration of 2.5% meat and bone meal in a feed sample

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SPECIFICITY OF MELISA-TEK® SPECIES KITS

SAMPLE TYPE	MELISA-TEK KIT REACTION		SAMPLE TYPE	MELISA-TEK KIT REACTION	
	PORK	RUMINANT			
Muscle Tissue (100°C-138°C)			Matrix		
Beef	-	+	Wheat Bran	-	-
Pork	+	-	Wheat Germ	-	-
Chicken	-	-	Oat Bran	-	-
Sheep	-	+	Corn Meal	-	-
Horse	-	-	Cane Sugar	-	-
Deer	-	-	Powdered Cow's Milk	-	-
Rabbit	-	-	Cooked Whole Egg	-	-
Water Buffalo	-	+	Peanut	-	-
Meat and Bone Meals			Soy Flour	-	-
AM1 (Beef & Pork Reference)	+	+	Matrix		
AM2 (Beef & Pork Reference)	+	+	Pork Blood	-	-
EU Feed Base Composite	-	-	Beef Blood	-	-
Commercial Feeds			Beef Gelatin	-	-
Calf Milk Replacer	-	-	Pork Gelatin	-	-
Cattle Herd Growth Feed	-	-	Beef Liver	-	-
Cattle Breeding Feed	-	-	Beef Lung	-	-

Limitations of the MELISA-TEK® Species Kits:

Numerous organic and inorganic compounds commonly found in food products and animal feeds have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in cooked meat and feed products, test interferences caused by matrix effects can't be completely ruled out. Mistakes in test handling and performance, including improper kit storage, pipetting errors, long or short incubation times, and temperature extremes during testing (less than 10°C or higher than 30°C) can also lead to erroneous results.

DISCLAIMER:

ELISA Technologies 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 ELISA Technologies discretion.

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REVISION DETAILS

Revised *MELISA-TEK Kit Instructions*
Revision No: 70308-v1

**** THE INSTRUCTION BOOKLET ****
HAS BEEN CHANGED
TO MAKE IT EASIER TO USE

**** THE TEST PROCEDURES ****
HAVE NOT CHANGED

The MELISA-TEK Kit instructions have been simplified and revised to be generic for the:

MELISA-TEK® RUMINANT KIT - Cat. No. 510311
MELISA-TEK® PORK KIT - Cat. No. 510321

The *MELISA-TEK* Ruminant and Pork follow the same protocols. The protocol has not changed and the new booklet is intended to make the instructions simpler and easier to use as well as allowing an assay to be configured to run both ruminant and pork simultaneously .

The note in the sample preparation section (page 6) refers to the ***ELISA-TEK HIGH SENSITIVITY EXTRACTION KIT – Cat. No. 510391*** which is now available and can be used as an alternative sample extraction procedure with the MELISA-TEK Species Kits. Use of the high sensitivity sample extraction procedure will increase the sensitivity of the MELISA-TEK assays approximately 5x with MBM and feed samples and allowing for detection of 0.1% Ruminant or Pork MBM in an animal feed.

Revision 110303-v2: Added percentage information to preservatives used in Species Muscle Tissue Control, Biotinylated Anti-Species Antibody and Wash Buffer Concentrate

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SPECIES TEST KITS AVAILABLE FROM

ELISA Technologies, Inc.

MELISA-TEK® COOKED MEAT SPECIES KITS **CAT NO:**

MELISA-TEK® RUMINANT (Bovine/Ovine) KIT	510311
MELISA-TEK® PORK KIT	510321
MELISA-TEK® HIGH SENSITIVITY EXTRACTION KIT	510391

ELISA-TEK® COOKED MEAT SPECIES KITS **CAT NO:**

COOKED MEAT MIXED KIT (Custom Kit)	510601
COOKED MEAT 3 Species (Beef,Pork,Poultry)	510603
COOKED MEAT 4 Species (Beef,Pork,Poultry,Sheep)	510604
COOKED MEAT BEEF KIT	510611
COOKED MEAT PORK KIT	510621
COOKED MEAT POULTRY KIT	510631
COOKED MEAT SHEEP KIT	510641
COOKED MEAT HORSE KIT	510651
COOKED MEAT DEER KIT	510661

ELISA-TEK® M&BM THERMAL EVALUATION KITS **CAT NO:**

Meat & Bone Meal Heat Control - PORK KIT	510629
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ELISA-TEK® RAW MEAT SPECIES KITS **CAT NO:**

RAW MEAT MIXED KIT (Custom Kit)	510501
RAW MEAT 3 SPECIES KIT(Beef,Pork,Poultry)	510503
RAW MEAT 4 SPECIES KIT(Beef,Pork,Poultry,Sheep)	510504
RAW MEAT COW KIT	510511
RAW MEAT PIG KIT	510521
RAW MEAT POULTRY KIT (Detects all poultry)	510531
RAW MEAT SHEEP KIT	510541
RAW MEAT HORSE KIT	510551

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