MELISA-TEK®

SPECIATION KITS FOR MEAT & BONE MEALS AND ANIMAL FEEDS

For the Qualitative 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 – Catalog # 510311

MELISA-TEK® PORK KIT – Catalog # 510321

ELISA Technologies
Committed to food and feed safety

MELISA-TEK®

SPECIATION KITS FOR MEAT & BONE MEALS AND ANIMAL FEEDS

CONTENTS

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

INTRODUCTION

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.

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.

PRINCIPLE OF THE TEST

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.

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.

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

KIT COMPONENTS

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.

B. **TWO** vials of **BIOTINYLATED ANTI-SPECIES ANTIBODY** containing 3.0 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 4.0 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 SUBSTRATE** containing 6.0 mL buffered and stabilized TMB (Tetramethylbenzidine).

F. **ONE** vial of **STOP SOLUTION** containing 6.0 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 EXTRACTION BUFFER PACKET** containing a mixture of phosphate saline buffer salts and EDTA sufficient to make 1.8 liters of **EXTRACTION SOLUTION**.

I. **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 their respective labels. Exposure of the kit and kit components to ambient or elevated temperatures (>2-8°C) should be minimized.

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

KIT STORAGE INSTRUCTIONS

***MELISA-TEK*® 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).

MATERIALS REQUIRED BUT NOT PROVIDED

1. Purified water.
2. Precision micropipettors and tips with a range of 20-1000 microliters.
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. 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. Plate or strip washer.
11. Plate reader with a 450 nm filter.

PROCEDURAL NOTES AND PRECAUTIONS

1. Review the complete Instructions for Use before performing the ***MELISA-TEK*®** assay.

2. ***MELISA-TEK*® 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.

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

7. Once 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. 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.

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 the enclosed worksheet template showing the 96 well layout. Mark the location of the wells selected for each control and sample extract on the Plate plan worksheet.

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

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 1.8 liters of purified water. Mix until contents are completely dissolved. The buffer may be stored at room temperature or refrigerated for the life of the kit.
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 × *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, Catalog Number 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 µL of the appropriate supplied 10% positive control to 900 µL of Extraction Solution. Mix gently by pipetting.
    ii. To prepare the 0.05% Low Positive Control, add 50 µL of the 1% High Positive Control (prepared above) to 950 µL 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*.

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

ASSAY PROCEDURE

1. Pipette 100 µL 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 50 µL of Biotinylated Secondary Antibody into each well.
5. Incubate 20 minutes at room temperature.
6. Wash the wells 3 times.
7. Pipette 50 µL of Avidin-Peroxidase Solution into each well.
8. Incubate 20 minutes at room temperature
9. Wash the wells 6 times.
10. Pipette 50 µL of TMB Substrate into each well.
11. Incubate 20 minutes at room temperature – DO NOT WASH.
12. Pipette 50 µL of Stop Solution into each well.
13. Read the plate on a 96-well plate reader with a 450 nm filter. Read the plate within 10 minutes of adding stop solution

DETERMINATION OF RESULTS

1. Take the mean of the triplicate values for the blank and each sample and control. This can be done on the worksheet provided.

2. Subtract the mean blank O.D. from the mean O.D. of each sample and control, and record these blank-corrected values on the worksheet.

3. The assay is considered **VALID** if:

    a. The mean blank-corrected O.D. of the 1% High Positive Control is greater than 1.000
    AND
    b. The mean blank-corrected O.D. of the 0.05% Low Positive Control is greater than 0.100
    AND
    c. The mean blank-corrected O.D. of the 10% Negative Control is less than or equal to 0.100
    AND
    d. The standard deviation of the 0.05% Low Positive Control replicates is less than or equal to 0.100

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

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

6. Test Sample Classification:
    a. Test samples are considered **POSITIVE** if the mean blank-corrected O.D. is GREATER than 0.100 and the assay is valid according to the criteria listed above.
    b. Test samples are considered **NEGATIVE** if the mean blank-corrected O.D. is LESS than or equal to 0.100 and the assay is valid according to the criteria listed above.

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

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 for 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 for 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

SPECIFICITY OF *MELISA-TEK®* SPECIES KITS

SAMPLE TYPE	**MELISA-TEK® KIT REACTION**	
Muscle Tissue	**PORK**	**RUMINANT**
Beef	-	+
Pork	+	-
Chicken	-	-
Sheep	-	+
Horse	-	-
Deer	-	-
Rabbit	-	-
Water Buffalo	-	+
Meat and Bone Meals		
AM 1 (Beef and Pork Reference)	+	+
AM 2 (Beef and Pork Reference)	+	+
EU Feed Base composite	-	-
Commercial Feeds		
Calf Milk Replacer	-	-
Cattle Herd Growth Feed	-	-
Cattle Breeding Feed	-	-

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

MELISA-TEK®

SPECIATION KITS FOR MEAT & BONE MEALS AND ANIMAL FEEDS

SAMPLE TYPE	MELISA-TEK® KIT REACTION	
Matrix	**PORK**	**RUMINANT**
Wheat Bran	-	-
Wheat Germ	-	-
Oat Bran	-	-
Corn Meal	-	-
Cane Sugar	-	-
Powdered Cow's Milk	-	-
Cooked Whole Egg	-	-
Peanut	-	-
Soy Flour	-	-
Matrix		
Beef Blood	-	-
Pork Blood	-	-
Beef Gelatin	-	-
Pork Gelatin	+	-
Beef Liver	-	-
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.

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

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 ELISA Technologies, Inc. discretion.

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

REVISION NOTES

140601: Replaced old logo with new logo, changed “ELISA” to bold font, changed preparation of extraction buffer.
141102: Changed picture to new kit box, changed amount of biotinylate bottles from 3 bottles of 2 mL each to 2 bottles of 3 mL each.
150715: Edited for clarification.

MANUFACTURED BY:

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

rev. 150715