

MELISA-TEK[®] High Sensitivity Extraction Kit (ETI #510391)

In order to achieve a higher level of sensitivity in the detection of troponin-I, the following protocol can be used in conjunction with the MELISA-TEK[®] Ruminant Kit (Catalog #510311). This high-sensitivity protocol allows for the detection of MBM at 0.1% (w/w) in feeds, feed bases and meals.

The high-sensitivity protocol differs from the standard protocol in terms of the sample preparation and instrument requirement. The instrument required (not provided) is a refrigerated centrifuge able to accommodate 15 mL centrifuge tubes and operate at 7000 $\times g$ for up to 60 minutes (e.g., Sorvall RC5C Plus).

Kit Components:

24 funnels	24 pleated filter papers	1 instruction sheet
24 concentrator tubes	24 pipette tips (for use with concentrator tubes)	

Protocol:

For all steps other than sample preparation, follow the Instructions for Use included with the MELISA-TEK[®] Kit. **The following section on sample preparation should replace the sample preparation instructions in the Instructions for Use** (page 6, under **Test Preparation**, item 2a-g):

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 with occasional mixing.
- 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 samples to cool for 30 minutes with occasional mixing.
- f. Filter the extract liquid using the fast-flow filter paper and funnels provided. The filtered extract may be clear or cloudy.
- g. Place 4 mL of the filtered sample extract into the protein concentrator.
- h. Centrifuge at 7000 $\times g$, 4°C for 20-60 minutes, or until the volume of the concentrate (held in the upper chamber of the concentrator) reaches 0.5 mL. If the sample concentrates beyond this point, bring the volume back up to 0.3-0.5 mL using the Extraction Solution prepared as described in the kit instructions, and pipette the concentrate up and down to mix.
- i. Mix and collect the sample concentrate using the pipette tips provided (which fit a standard P1000 pipettor). No further processing is required before pipetting the concentrated sample onto the plate.



ELISA Technologies
Committed to food and feed safety

REVISION NOTES

160309: Added revision number, footer, and manufacturer information

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Rev. 160309