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User's Manual

Horse Meat Adulteration ELISA Kit

REF **DEIASL023**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is used for Horse Meat identification or adulteration detection in raw, uncooked meat or grounded meat.

General Description

The 2013/2014 meat adulteration scandal is ongoing in Europe; foods advertised as containing beef were found to contain undeclared horse meat, as much as 100% of the meat content in some cases, and other undeclared meats, such as pork. The issue came to light on 15 January 2013, when it was reported that horse DNA had been discovered in frozen beef burgers sold in several Irish and British supermarkets. It is speculated that Donkey and Mule may have been used in tainted beef or pork. While horse meat is not harmful to health and is eaten in many countries, it is considered a taboo food in many countries including the UK and Ireland. As horses are relatively poor converters of grass and grain to meat compared to cattle, they are not usually bred or raised specifically for their meat. Instead, horses are slaughtered when their monetary value as riding or work animals is low, but their owners can still make money selling them for horse meat. Therefore, horses, donkeys or mules used may not be from 'healthy herds'. People are also concerned about the presence of some drugs used in animals that are banned for human use. The presence of many animal viruses of diseases in non-approved, unhealthy animals is an issue as well. Some of the largest meat suppliers in Europe (TESCO, LIDL, Iceland, ALDI, Ikea etc) are involved in meat adulteration. Horse meat found its way into popular fat food market (Taco Bell, Burger King etc, school lunches, and hospital food. Jewish and Muslims religion prohibit eating horses. Adulteration of beef or chicken with pig is also a problem for Muslims, whereas Hindu religion prohibits the meat of the Cow or Beef. Regardless of the ethics or religious concerns, it is simply unethical to sell horse meat that is labeled as beef. A huge stock of unsold beef, pork, and chicken has been recalled due to the concerns of horse meat adulteration resulting into millions of dollar in monetary damage. Many consumers have also lost trust in the meat industry and stopped buying meat.

Principles of Testing

Horse meat identification or adulteration ELISA kit is based on sequential binding of proteins found in Horse meat to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme HRP. After a washing step, chromogenic substrate (TMB) is added and colors (blue) developed. The enzymatic reaction (color) is directly proportional to the amount of hrose meat present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

Reagents And Materials Provided

1. Anti-Horse meat specific proteins antibody coated microwell strip plate (black line of strips): 12 strips
2. Horse Meat proteins Negative control: 1 vial
3. Horse Meat proteins Positive control: 1 vial

4. Meat Proteins Extraction buffer (100X): 5 mL
5. Horse meat protein antibody-HRP Conjugate: 12 mL
6. Wash Buffer (100X): 10 mL
7. HRP substrate solution: 12 mL
8. Stop solution: 12 mL
9. Meat Spatula: 96pcs
10. Complete Instruction Manual

Materials Required But Not Supplied

1. Disposable Meat sample tubes (5-ml)
2. Disposable pipette tips (1-200 ul)
3. Pipette set (contain 5 pipettes of 5, 10, 20, 50, and 100 ul)
4. Meat sample spatulas (individually wrapped)
5. 50 and 500 ml graduated tubes or containers.
6. Paper towels
7. Distilled or deionized water
8. ELISA reader and washer (optional).
9. Washing manifold or ELISA washer and ELISA reader

Storage

This kit is stored at 2 ~ 8 °C, protect from light.

Specimen Collection And Preparation

1. Fresh or frozen and uncooked meat-if meat is frozen then it should be thawed at 4°C or room temp until it can be cut into

pieces. Big chunks should be cut into small pieces such as found in grounded meat. If the meat is grounded then it can be used directly. Note: DO NOT wash the meat to remove blood, it is necessary to have the blood traces in the meat as it also contains meat proteins.

2. Take a small amount of meat (~100 mg) sample using the spatula provided in the kit. If necessary, take several portions of the meat samples from different places the packaged meat and mix in a clean water cup or sample cup or meat paper (not provide). Mix the meat portions and then take a small amount of meat. This is to assure that you get a good representation of the meat contents.
3. Transfer the meat sample into a clean sample tube. Add 2-ml of diluted meat extraction buffer (note: extraction buffer is provide 100x and it must be diluted prior to use; Mix vigorously and manually and let the tube incubate for at least 30-mins. If a shaker is available then the tubes can be left on the shaker. It is also possible to extract the samples overnight at 4°C for convenience.
4. Let the meat sample tube sit for 5-10 mins to allow meat residue settle at the bottom. Carefully transfer about 1 ml clear liquid using a pipette or carefully pour into a separate 1-2 ml tube (not provided). Clear top

liquid (Extracted samples) will be used for testing. The samples are 5% (w/v; or 1:20 diluted). Extracted samples can be stored at 4°C for up to 1-week or stored frozen at-20°C or below for 6-months until tested.

5. Extracted samples (1:20 as above) can be tested undiluted or diluted 1:1000 in extraction buffer (dilute 20 ul of sample and 1 ml of buffer).

Reagent Preparation

Dilute 100X wash buffer 1:100 with water (5 ml stock in 500 ml distilled or deionized or bottled drinking water). Store at 4°C

until needed.

Dilute Meat Proteins Extraction buffer (Pink color) 1:100 with water (1ml stock in 100 ml distilled or deionized or bottled

drinking water). Store at 4°C until needed.

Assay Procedure

Arrange required # of strips. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Prepare 1x wash buffer and meat extraction buffer by diluting 1:100 with water (dilute 1 ml in 100 ml distilled or deionized water). Store at 4°C until use.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipete 100 ul of supplied negative and positive controls into the designated wells. Add 100 ul of extracted meat samples (1:20 or 1:1000) into appropriate wells. Mix gently for 5-10 seconds by tapping the plate against the palm, cover the plate and incubate at room temp. for 30 minutes.
3. Aspirate and wash the wells 3 times with 1X wash buffer. Use 300 ul per well or use supplied 8-well manifold.

Transfer the 1x wash buffer into v-shaped tray and add about 300 ul per wash. If using few wells then it is possible to add 300 ul wash buffer directly into wells. Shake the plate for a few seconds and then dump the liquid into waste container. Repeat the wash as required. After the last wash, plate must be tapped over paper towel between washings to ensure proper washing. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values.

4. Add 100 ul of antibody enzyme conjugate into each well. Mix gently for 5-10 seconds, cover the plate and incubate at room temp. for 20 minutes.
5. Wash the strips 4X as in step 3. After the last wash, tap the strips over the fresh paper towels to remove traces of conjugate.
6. Add 100 ul of TMB substrate into all wells. Mix gently for 5-10 seconds and incubate for 10 mins at room temp. Positive wells will develop blue color.
7. Add 100 ul stop solution into each well (blue color turns into yellow). Read absorbance at 450 nm using an ELISA reader within 30 minutes.
8. Results recorded visually and picture taken or read A450nm of the ELISA plate for data analyses.

Quality Control

Kit supplied negative and positive controls and know negative and positives controls (user supplied) must be run with every test. Negative controls values must be A450=1.00 or dark light blue/yellow). Repeat the test if test failed to meet these guidelines.

High blank values (negative control A450=>0.500 or dark blue/yellow) are due to insufficient washing of the wells.

Increase the number of washing and repeat the color until acceptable values are met.

Interpretation Of Results

Negative samples: Very light blue/yellow color obtained as in supplied negative horse meat control. Samples do not contain detectable horse meat proteins or samples considered to be horse meat free or no detectable horse meat adulteration.

Positive samples: Dark light blue/yellow color obtained as in supplied Positive horse meat control. Samples contain detectable horse meat proteins at 1% or higher; samples considered to be horse meat origin or have significant horse meat adulteration.

All critical samples must repeated to confirms the results and also tested with an alternative test. As with any other tests, no single tests should be seen as 100% confirmatory.

Sensitivity

Ground horse meat proteins were extracted and tested at various concentration (w/v %). Horse meat proteins were tested in the kit using 5%, 0.5%, 0.05%, 0.005%, and 0.0005% (v/v) samples. The sensitivity of the kit has been assessed as 0.005% (1 part per million or 1 ppm).