

ELISA kits available from ADI (see details at the web site)

Catalog# Product Description

BMT-24 1-hour Beef/Cow meat adulteration ELISA test

DMT-24 1-hour Dog meat adulteration ELISA test (

HMT-24 1-hour Horse meat adulteration ELISA test

BMT-24 1-hour Pig meat adulteration ELISA test

RMT-24 1-hour Rat meat adulteration ELISA test (for beef or pig meat; 24 tests)

MSSW-100 Meat sample spatulas (individually wrapped) 100/pack

MSSW-1000 Meat sample spatulas (individually wrapped) 1000/pack

MST5-1000 Disposable Meat Sample Plastic tubes with caps (5 ml)
(1000/pack)

MST5-250 Disposable Meat Sample Plastic tubes with caps (5 ml)
(250/pack)

YPT96-1 Disposable yellow pipette tips (1-200 ul) for meat sample (1 box 96 tips)

YPT96-10 Disposable yellow pipette tips (1-200 ul) for meat sample (10
box 96 tips)

New Products

Rapid Tests or lateral flow tests for beef, pig, chicken, horse, and dog meat adulteration or species identification

Simple dipstick test to give positive or negative test results in 5-10 minutes. No lab reagents are required.

Instruction Manual No. BMT-24

Bovine/Cow Meat Identification ELISA Kit

Cat # BMT-24 (24 tests)

Cat # BMT-48 (48 tests)

Cat # BMT-96 (96 tests)

1-hour Bovine/Cow Meat identification or adulteration detection in raw, uncooked meat or grounded meat



For In Vitro Research Use Only



**ALPHA DIAGNOSTIC
INTERNATIONAL**

6203 Woodlake Center Drive • San Antonio • Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Bovine/Cow Meat Identification ELISA KIT

Kit Contents: #BMT-24 (24 tests), #BMT-48 (48 tests), #BMT-96 (96 tests)

Components	Qty
Anti-Bovine/Cow meat specific proteins antibody coated microwell strip plate (Brown color lines on the strips) #BMT241	3 strips (BMT-24) 6 strips (BMT -48) 12 strips (BMT -96)
Bovine/Cow Meat proteins Negative control (<i>blue cap</i>); #BMT-NC, 2 ml	1 vial
Bovine/Cow Meat proteins Positive control (red cap); #BMT-PC, 2 ml	1 vial
Meat Proteins Extraction buffer (pink solution ; green dot); (100X) #MEB-100	2 ml (BMT-24) 2 ml (BMT-48) 5 ml (BMT-96)
Bovine/Cow meat protein antibody-HRP Conjugate (yellow dot) #BMT24-PAC	3 ml (BMT-24) 6 ml (BMT-48) 12 ml (BMT-96)
Wash Buffer (100X) , #WB-100 (<i>blue cap</i>), 10 ml	1 bottle
HRP substrate Soln , Brown bottle, #80091	4 ml (BMT-24) 8 ml (BMT-48) 12 ml (BMT-96)
Stop solution # 80101, 12 ml (red cap)	1 bottle
Meat Spatula # MSSW	24, 48, or 96
Complete Instruction Manual	BMT-24

Intended Use

ADI's 1-hour meat adulteration or contamination ELISA test is designed to test uncooked, fresh Bovine/Cow meat or ground meat (fresh or frozen) for the presence of Bovine/Cow specific meat proteins. The kit is specifically formulated to be used in non-laboratory or field setting without the use the sophisticated lab equipment or personnel. The test provides a visual color (blue and yellow) in 1-Hour. *For in vitro research use only.*

The 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.

PERFORMANCE CHARACTERISTICS

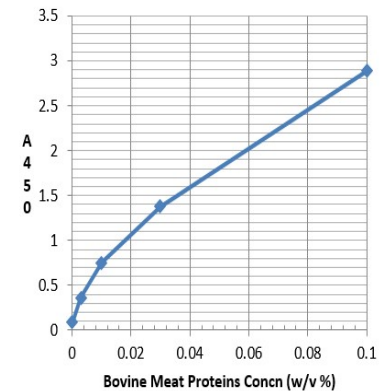
Quality Control

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 $<A450=0.300$ or very light blue/yellow). 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.

Sensitivity of Test

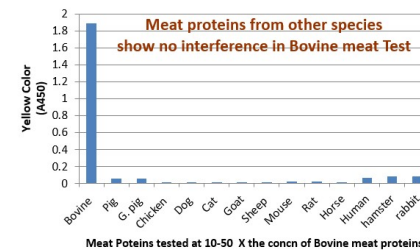
Ground Bovine/Cow meat proteins were extracted and tested at various concentration (w/v %). Bovine/Cow meat proteins were tested in the kit using 5%, 0.5%, 0.05%, 0.005%, and 0.0005% (v/v) samples. The sensitivity of the kit has been assessed at 1 part per million or 1 ppm



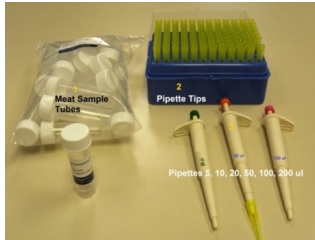
/6_ADI_ELISA

Species Interference

Antibodies used in the kit are specific for proteins found in Bovine/Cow meat. Similar proteins from many other common species did interfere when tested 50-100 fold higher concentration than Bovine/Cow meat.



Items required but not supplied



1. Disposable Meat sample tubes (5-ml), Cat #MST5-250 (pack of 250)
2. Disposable pipette tips (1-200 ul), Cat#YPT200-1 (box of 96)
3. Pipette set #SCMP-Set5 (contain 5 pipettes of 5, 10, 20, 50, and 100 ul)
4. Meat sample spatulas (individually wrapped), # MSSW-100 (100/pk); MSSW-500 (500 pk)
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

Most labs may already have these common lab items but they can be purchased separately if not available.

Make ready to use reagents and assemble other accessories (not supplied with the kit)

Accessories required but not supplied

See page 2...you must have all of these before you prepare the reagents kits or samples.

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 4oC until needed.

Dilute Meat Proteins Extraction buffer (Pink color) 1:100 with water (1 ml stock in 100 ml distilled or deionized or bottled drinking water). Store at 4oC until needed.



SPECIMEN COLLECTION AND HANDLING

1. **Fresh or frozen and uncooked meat**-if meat is frozen then it should be thawed at 4oC or room temp until it can be cut into pieces. Big chunks should be cut into small pieces such as found in ground meat. If the meat is ground 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 as shown above in Pic. 1. 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; Pic 2.). 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 4oC 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 (Pic 3). The samples are 5% (w/v; or 1:20 diluted). Extracted samples can be stored at 4oC for up to 1-week or stored frozen at-20oC 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).

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE). **If you have not used this kit before, we recommend to use 1 strip to run the controls alone to get familiar with the test and not run the risk of making mistakes and lose sample or the whole kit.**

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 4oC until use.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **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 (see Pic 4, page 3). 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.

WORKSHEET OF TYPICAL ASSAY

	1	2	3
A	-ve (0.100)	S7	S15
B	+ve (1.500)	S8	S16
C	S1	S9	S17 (1.450)
D	S2	S10	S18
E	S3 (1.100)	S11	S19
F	S4	S12	S20 (1.87)
G	S5	S13 (1.200)	S21
H	S6	S14	S22



NOTE: A typical arrange of controls and samples (S1-S22) placement is shown above. **Blue color** develops in positive controls and samples and **it turns yellow** upon addition of the stop solution. Values in parenthesis are A450 reading after stop.

Interpretation of Results

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

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

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.

In May, 2013, Rat meat found its way in meat sold as mutton or lamb in China (<http://blogs.wsj.com/chinarealtime/2013/05/03/rats-china-chews-on-new-food-safety-scandal/> also see June 2014 update: <http://www.usatoday.com/story/money/business/2014/07/22/china-meat-scandal-hits-starbucks-burger-king/12983025/>). It is possible that other animals such as Dog and Cat meat may have also been used for adulteration of meat for human or animal food.

The only way to resolve the issues is to have simple, easy, inexpensive meat adulteration test available that can be used by the meat suppliers, sellers and consumers. ADI's 1-hour horse, Beef, Pig, and Rat meat adulteration ELISA field test should be very useful in testing large number of samples and restoring the public confidence.

The kit is specifically formulated to be used in non-laboratory or field setting without the use the sophisticated lab equipment or personnel. The test provides a visual color (blue and yellow) in 1-Hour. Test Results can be photographed or reading recorded using ELISA reader to determine the % meat or other meat contamination. This kit can be used at meat processing plants, bulk meat buyers or sellers to perform quick and independent testing of meat samples without the hassle and cost of sending samples to an outside labs or waiting for results. Any positive samples by 1-Hr ELISA tests can be further confirmed by DNA or other tests. In this way, bulk of the meat testing cost and sample delays can be minimized. Therefore, 1-Hr ELISA Field test clearly offers advantages over other tests. In comparison to horse DNA test, ADI's 1-hour meat protein ELISA test is far superior due to the high sensitivity (1 part per million for ELISA vs 1 part per 10,000 For DNA), speed (1 hr Vs many hours to days), effectiveness (process large # of samples) and the overall cost of a ELISA field test is much less than the total cost of DNA tests.

The availability of a rapid, sensitive, and inexpensive meat field test will help control the meat adulteration. Separate meat tests kits are also available to test the presence of pig, beef, chicken, goat, cat, dog meat proteins.

PRINCIPLE OF THE TEST

Bovine/Cow meat identification or adulteration ELISA kit is based on sequential binding of protein found in Bovine/Cow 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 Bovine/Cow 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.

MSDS

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10