1-hour Horse, Pig, Cow/Beef, and Rat Meat Adulteration ELISA Test

ADI's 1-hour horse meat adulteration or contamination ELISA test is designed to test the ground beef or pig meat or horse (fresh or frozen) for the presence of horse meat. ***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 % horse meat or other meat contamination.*

ADI's 1-Hour Horse, Pig, or Beef Adulteration ELISA is meant to be used in **field setting** (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 horse 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.

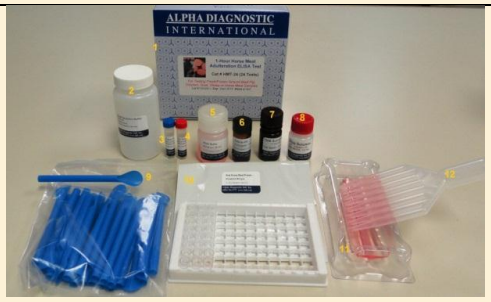
The availability of a rapid, sensitive, and inexpensive horse meat field test will help control the meat adulteration. Separate meat tests kits are also available to test the presence of pig, beef, chicken, goat, cat, dog meat proteins.

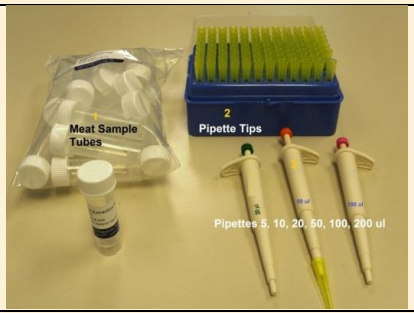
Key features of "1-Hour Horse Meat Adulteration ELISA test

- Kit contains all necessary critical reagents to complete the test in 1-hour with no lab equipment or instruments.
- Microwell ELISA test strips (24/48/96 wells), coated with proprietary antibodies to proteins found in horse meat
- Horse meat proteins Negative Control & Horse meat proteins positive controls
- Samples: Fresh or frozen meat grounded/sliced/patties(about 100 mg or a small meat fragment)
- The test produces blue/yellow color for positive samples.
- Sensitivity at 1 ppm (1 part per million) or 0.0001% (w/v).
- No interference from beef, pig, chicken, dog, cat, goat, sheep, mouse, rat and human meat proteins.
- Conveniently packaged in 24, 48 or 96 tests
- Kits are shipped at ambient or cold packages and stored at 4oC. Shelf life at least 6-months.

Test Description	24 tests cat#	48 tests cat#	96-tests cat#
1-hour **Horse meat** adulteration ELISA test	# HMT-24	# HMT-48	# HMT-96
1-hour **Pig meat** adulteration ELISA test	# PMT-24	# PMT-48	# PMT-96
1-hour **Bovine/Cow meat** adulteration ELISA test	# BMT-24	# BMT-48	# BMT-96
1-hour **Rat meat** adulteration ELISA test	# RMT-24	# RMT-48	# RMT-96
Accessories not supplied with the kit Disposable Meat Sample Plastic tubes (5 ml)	#MST5-250 (250/pk); #MST5-1000 (1000/pk)		
Disposable yellow pipette tips (1-200 ul); #YPT96-1 1 box of 96; #YPT96-10,10 boxes of 96			
Single channel fixed volume pipette set (5, 10, 20, 50, 50, 100 ul)	#SCMP-Set5		
Meat sample spatulas (individually wrapped)	MSSW-100 (100/pk); MSSW-1000 (1000 pk)		

1-Hour Horse Meat Adulteration ELISA Test Components





India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@atzlabs.com Website: www.atzlabs.com

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1-Hour Horse Meat Adulteration ELISA test Kit supplied Components

1. Kit Box containing all necessary reagents
2. Meat Sample Buffer
3. Horse meat protein Negative Control
4. Horse meat protein Postive Control
5. Wash Buffer
6. Antibody-HRP Conjugate
7. HRP-coloring reagent
8. Color Stop Solution
9. Meat Sample spatulas
10. Horse Meat Protein Antibody Coated Strips of 8-wells
11. Wash buffer tray
12. 8-well wash buffer manifold

Accessories 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. Horse meat sample spatulas (individually wrapped)
   # MSSW-100 (100/pk); MSSW-500 (500 pk)

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

1-Hour Horse Meat Adulteration ELISA Test-Brief Procedure

Step 1. Take a sample of freshly grounded beef (or other meats) using supplied disposable plastic meat sample spatula. Take a small fragment as shown in the **Pic. 1**. Frozen meat samples should be thawed at room temp for 30 mins-1 hr.

Step 2. Transfer the meat sample to the meat sample tubes as shown in **Pic 2**. Add 2-ml of the supplied meat sample buffer to the tube, close the cap, and mix vigorously for 30 seconds by hand and leave at room temperature for 1-2 hours.

Step 3.1. Transfer the **100ul of negative control** (-ve) and horse meat **positive control** (+ve, supplied), **extracted meat sample** solution to the coated plate (supplied in 8-wells strips) as shown in **Pic 3**. Use the appropriate # of strips and wells as needed.

Step 3.2. **Leave the controls and the sample in the plate for 30 mins at room temp** (25-28oC). After incubation, discard the plate contents and wash the wells with the supplied wash buffer (pink solution) using the wash buffer tray and the 8-well manifold (supplied). **Wash 3-times using 300-400 ul wash buffer per well (Pic 4).**

Step 3.3. Add 100-ul of the antibody-HRP conjugate to all wells using the 100-ul pipette. Leave the conjugate on the plate (incubation) at room temp for 20-mins. Wash the test strips with the wash buffer solution 4-times as in step 3.2.

Step .4. **Add 100 ul of clear coloring reagents to all wells** using 100-ul pipette and briefly mix the plate manually for 5-10 secs. **Blue color appears in +ve controls and the positive sample**. **Develop color for 5 mins** at room temp (**Pic 5**).

Step 5. Stop color by adding 100-ul of the stop solution to all wells (**blue color turns yellow**). Yellow color is stable for several hours (**Pic 6**).

Record the results by visual comparison of the +ve controls and the samples. A picture of the plate can be taken or plate read at 450 nm using standard ELISA reader that converts the color into A450 reading (0.00-3.00). Intensity of the yellow color is directly proportion to the concentration (% horse meat) in the sample. Higher or intense yellow color means more horse meat in the sample. **Horse meat contamination (%)** can be assessed by comparing the color intensity (visually) or using the A450 ELISA reader values by running horse meat controls at 100%, 50%, 10%, and 1%. Similar meat contamination or adulteration ELISA kits is also available for pig and cow (bovine or beef).

Performance Characteristics of the 1-Hour Horse Meat Adulteration ELISA test

Quality Controls

Supplied negative and +ve controls must be run in every test and they should remain –ve and +ve as per the guidelines for the test to be valid. Ground Beef samples from local San Antonio, Texas, Supplier (HEB) shown not to contain any horse or pig meat. Similarly ground Pig meat sample was negative for horse and beef.

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 at 1 part per million or 1 ppm (Fig. 1)

Species Crossreactivity (false positive)

Horse meat protein ELISA test is highly specific for horse proteins with no interference from other meat proteins from a variety of animals (Fig. 2).

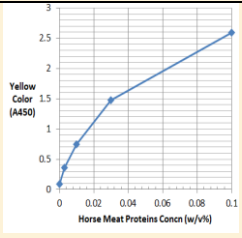

Fig. 1. A typical standards curve for horse protein found in meat. Sensitivity is ~1 ppm.

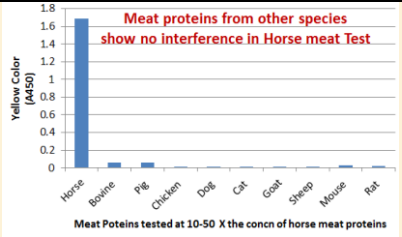

Fig. 2…Horse meat protein ELISA test is specific for horse with no significant interference from meat proteins from bovine, pig, chicken, dog, cat, goat, sheep, mouse, rat, and human. All species reactivity was negligible at 0.01-0.2%).

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Key Feature of Horse DNA test and ADI's Horse Meat Protein's Test

Items	Horse DNA Test	1-hr Horse Meat Protein ELISA Test	Advantages of Horse Protein test
Recovery of testing agents	Average of 200 ug total DNA recovered per 100 mg tissue	Horse meat target protein ~200 mg protein in 5% horse meat	Target Protein is 1000-10,000 fold more abundant
Procedure	DNA Isolation, purification followed by PCR	Direct testing of horse protein extracts	Simple and rapid sample processing
Test Duration	1-3 days	1 hour	Horse Protein Test is extremely rapid
Test complexity	Extremely high; require sophisticated lab and highly trained personnel	Extremely simple; test performed even by non-scientists	Horse Protein test can be performed by most non-scientists
Cost	Several 100$ per test in reagent, equipment and personnel cost	$10 per test; $2-3 in bulk purchase	1-hr test is very cost effective
Automation or high thru put	best performed on individual samples	Up to 96 samples at a time	Save time with 1-hr test
Sensitivity	0.01% or 1 part per 10,000	0.0001% or 1 ppm	
On-site or field tests	No. DNA test can only be performed in well-equipped and experiences sHNcientists	Test supplies all reagents needed and it can be used on-site and visual results available in an hour	Horse Meat Protein test is more suitable for use by meat manufacturers, buyers, law enforcement agencies

Self-regulations, financial industry, meat industry, drug abuse by athletes or any other kind, is always prone to violations by a few but greedy exploiters. The exploitation is easier and more pronounced in the absence of an independent, quick and easy testing. The ongoing European 2013 horse meat adulteration of beef meat in Europe, once again, is the result of limited or no testing by the federal agencies. The scandal has results in huge financial losses by the meat suppliers due to recall of tainted beef products; the scandal has also damaged the trust of the consumers. The horse DNA test, currently in use, can only be performed by a very few expert laboratories; it is time consuming and very expensive. There are test results delays due to the collection, processing, and sending the samples to a few testing labs in Europe. There is an urgent need to develop a simple, rapid, and inexpensive test that can be performed at the grocery stores, meat packagers and the users of meat such as restaurants, fast food suppliers (McDonalds, Burger King, Taco Bell, etc).

ADI, a biotech company in San Antonio, Texas, USA, has developed the **first field test, 1-hour Horse Meat Adulteration ELISA Test**, which detects the **presence of horse meat-specific proteins** in grounded or sliced meat samples from horse, pig/pork, cow/beef, cat, dog, chicken, human, and sheep/goat. The horse meat protein adulteration test can be performed at home, restaurant, grocery store, qualified laboratories or law enforcement agencies. Meat samples (uncooked, grounded) using as little as a single meat fragment of the size of pencil head can be used. Horse meat test kit has all necessary reagents and no sophisticated lab instruments are used. The **test results are available in 1 hour with a visual blue yellow color development in supplied test strips**; it detects the horse meat protein contamination at 0.0001% or less. It is also possible to get an approximate % meat adulteration in samples by comparing the samples with known reference horse meat samples with adulteration at 1, 10%, 50%, and 100%.

2013 Horse Meat Scandal: The issue, business, ethics, and prevention of meat adulteration

The 2013 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.

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/). 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.

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