VERSION 1.01

NITROIMIDAZOLES ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Nitroimidazoles ELISA Test Kit

Catalogue Number. IP100025

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Nitroimidazoles in the sample. The coupling antigens are pre-coated on the micro-well stripes. The Nitroimidazoles in the sample and the coupling antigens pre-coated on the micro-well stripes compete for the anti-Nitroimidazoles antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Nitroimidazoles in it. This value is compared to the standard curve and the Nitroimidazoles concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.1ppb
Detection limit: Pork, Chicken about 0.5ppb, Honey about 0.1ppb, Raw milk (method 1) about 1ppb, Raw milk (method 2) about 0.2ppb, Raw egg (method 1) about 0.5ppb, Raw egg (method 2) about 1.5ppb, Cooked egg about 1ppb
Note: ppb= ng/ml or ng/g
Cross-reaction rate: Metronidazole 100%, Hydroxyl Nitroimidazoles 47%, Hydroxymethyl dimetridazole 53%, Tinidazole 2%, Dimetridazole 800%, Ornidazole 5%
Recovery rate: 90±30%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0ppb, 0.1ppb, 0.3ppb, 0.9ppb, 2.7ppb and 8.1ppb	
3	Enzyme conjugate	12ml	1 bottle
4	Antibody working solution	6ml	1 bottle
5	Substrate A	6ml	1 bottle
6	Substrate B	6ml	1 bottle
7	Stop solution	6ml	1 bottle
8	20× concentrated washing buffer	30ml	1 bottle
9	Redissolving solution	50ml	1 bottle
10	Tissue sample treatment solution Ⅰ (dry powder, optional)		1 bottle
11	Milk sample treatment solution (10ml, optional)		1 bottle

Materials required but not provided

Equipments: microplate reader (450nm, 630nm), printer, homogenizer, nitrogen-drying device, vortex, shaker, centrifuge (3000g and above), measuring pipets, balance (a reciprocal sensibility of 0.01 g), incubator (4℃, 25℃), water bath, timer;
Micropipettors: single-channel 20-200 µL, 100-1000 µL, and eight-channel 30～300 µl;
Reagents (AR): HCl, NaOH, ethyl acetate, n-Hexane.

Sample pre-treatment

Instructions

1) Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents;
2) Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.
Solution preparation before sample pre-treatment
1) Tissue sample treatment solution Ⅰ: dissolve the dry powder with 200ml deionized water(Ask supplier if needed).
2) Tissue sample treatment solution Ⅱ: mix 400ml ethyl acetate and 100ml n-Hexane.
3) Washing buffer: 1 part 20× concentrated washing buffer + 19 parts deionized water.
4) 0.1 M NaOH: dissolve 0.8g NaOH in 200ml deionized water.
5) 1M HCl: Weigh 1ml HCl, add 11ml deionized water to dissolve.

Samples preparation

a) Tissues (chicken, pork)

1) Weigh 2 g of the homogenized sample (tissue) into 50ml plastic centrifuge tube;
2) Add 2ml Tissue sample treatment solution Ⅰ, shake strongly for 1min (or use vortex for 30s), making Tissue sample treatment solution Ⅰ mix with sample completely;
3) Add 8ml Tissue sample treatment solution Ⅱ, shake or vortex for 3min (Note: if there is Shaker in lab, shake for 1min, then put it in Shaker at 300rpm at 25℃ for 10min);
4) Centrifuge at above 3000 g at room temperature for 5min;
5) Take 4ml supernatant into a clean glass centrifuge tube;
6) Blow to dry in 50-60℃ water bath by nitrogen-drying device;
7) Add 2ml n-Hexane, then 0.5ml Redissolving solution, shake strongly for 1min (or use vortex for 30s);
8) Centrifuge at above 3000 g at room temperature for 5min;
9) Discard up-layer n-Hexane and middle-layer Impurity layer;
10) Take 50ul down-layer liquid to test.
Fold of dilution of the sample: 0.5

b) Honey

1) Weigh 2± 0.05 g honey sample into 50ml plastic centrifuge tube;
2) Add 2ml 0.1M NaOH, shake strongly for 1min (or use vortex for 30s) to dissolve;
3) Add 4ml ethyl acetate, shake strongly for 5min (or vortex for 3min), making sample and ethyl acetate contact completely;
4) Centrifuge at above 3000 g at room temperature for 5min;
5) Take 2ml supernatant into 10ml glass tube(Note: do not take down-layer water phase), blow to dry in 50-60℃ water bath by nitrogen-drying device;
6) Add 0.5ml Redissolving solution, shake strongly for 2min (or use vortex for 1min);
7) Take 50ul down-layer liquid to test.
Fold of dilution of the sample: 0.5

c) Raw milk
Method 1

1) Take the collected raw milk , thaw and return to room temperature for above 30min;

2) Put tips into down-layer of raw milk, take out 1ml sample into 2ml centrifuge tube(Note: do not take up-layer cream);
3) Add 50ul 1M HCl, shake strongly for 1min (or vortex for 30s);
4) Centrifuge at above 4000 g at room temperature for 10min;
5) Take 100ul up-layer clear liquid into another centrifuge tube (do not take up-layer cream), then add 400ul Redissolving solution,shake strongly for 1min (or vortex for 30s);
6) Take 50ul liquid to test.
Fold of dilution of the sample: 5

Method 2

1) Take the collected raw milk , thaw and return to room temperature for above 30min;
2) Put tips into down-layer of raw milk, take out 2ml sample into 50ml centrifuge tube(Note: do not take up-layer cream);
3) Add 100ul Milk sample treatment solution, then add 6ml ethyl acetate, shake strongly for 5min (or vortex for 1min);
4) Centrifuge at above 3000 g at room temperature for 5min;
5) Take 1.5ml up-layer clear liquid into another clean glass centrifuge tube, blow to dry in 50-60℃ water bath by nitrogen-drying device;
6) Add 1ml n-Hexane, then add 0.5ml Redissolving solution,shake strongly for 1min (or vortex for 30s);
7) Centrifuge at above 3000 g at room temperature for 5min;
8) Discard up-layer n-Hexane and middle-layer Impurity layer;
9) Take 50ul down-layer liquid to test.
Fold of dilution of the sample: 1

d) Raw egg
Method 1

1) Take 1ml whole egg sample into 50ml plastic centrifuge tube;
2) Add 1ml 0.1M NaOH, 8ml ethyl acetate, shake strongly for 5min (or vortex for 3min),making sample and ethyl acetate contact completely;
3) Centrifuge at above 3000g at room temperature for 5min;
4) Take 4ml supernatant into a clean glass centrifuge tube(Note: do not take down-layer water phase); Blow to dry in 50-60℃ water bath by nitrogen-drying device;
5) Add 0.5ml Redissolving solution, 1ml n-Hexane, shake strongly for 2min (or use vortex for 1min);
Centrifuge at above 3000 g at room temperature for 5min;
6) Discard up-layer n-Hexane and middle-layer Impurity layer;
7) Take 50ul down-layer liquid to test.
Fold of dilution of the sample: 1

Method 2

1) Take 1ml sample into 2ml centrifuge tube;
2) Add 100ul 1M HCl, shake strongly for 1min (or use vortex for 30s);
3) Centrifuge at above 4000g at room temperature for 10min;
4) Take 100ul up-layer clear liquid into another clean glass centrifuge tube, add 400ul Redissolving solution, shake strongly for 1min (or use vortex for 30s);
5) Take 50ul liquid to test.
Fold of dilution of the sample: 5

e) Cooked egg

1) Take 1ml cooked egg sample into 50ml plastic centrifuge tube;
2) Add 1ml 0.1M NaOH, 8ml ethyl acetate, shake strongly for 5min (or vortex for 3min),making sample and ethyl acetate contact completely;
3) Centrifuge at above 3000g at room temperature for 5min;
4) Take 2ml supernatant into 10ml glass centrifuge tube(Note: do not take down-layer water phase); Blow to dry in 50-60℃ water bath by nitrogen-drying device;
5) Add 0.5ml Redissolving solution, 1ml n-Hexane, shake strongly for 2min (or use vortex for 1min);Centrifuge at above 3000 g at room temperature for 5min;
6) Discard up-layer n-Hexane and middle-layer Impurity layer;
Take 50ul down-layer liquid to test.
Fold of dilution of the sample: 2

ELISA procedures

Instructions

1) Bring all reagents and micro-well strips to the room temperature (20-25 ℃) before use;
2) Return all reagents to 2-8 ℃ immediately after use;
3) The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in ELISA the procedures;
4) For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation procedures

1. Bring test kit to the room temperature (20-25 ℃) for at least 30 min, note that each reagent must be shaken to mix evenly before use, put the required micro-well strips into plate frames. Re-sealed the unused microplate, store at 2-8 ℃, not frozen.
2. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
3. Add 50 µL of the sample or standard solution into separate duplicate wells; then add 50 µL of the antibody working solution into each well, mix gently by shaking the plate manually. Seal the microplate with the cover membrane, and incubate at 4 ℃ for 60min in dark.
4. Pour liquid out of microwell, flap to dry on absorbent paper, add 250 µL/well of washing buffer to wash microplate for 15-30 s, then take out and flap to dry with absorbent paper, repeat 3-4 times. (If there are the bubbles after flapping, cut them with the clean tips).
5. Add 100ul enzyme conjugate, mix gently by shaking the plate manually(After washing plate, do not put it aside for a). Seal the microplate with the cover membrane, and incubate at 25 ℃ for 20min in dark. Washing as step 4.
6. Coloration: add 100ul mixture of substrate A solution and substrate B solution into each well (Note: mix substrate A solution and substrate B solution at 1:1, use the mixture in 10min, do not use metal to contain or stir, to avoid substrate invalid). Mix gently by shaking the plate manually, seal the microplate with the cover membrane then incubate at 25 ℃ for 15 min at dark for coloration.
7. Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Stop successfully when substrate color from blue to yellow. Recommend to read the OD value at the dual-wavelength 450/630 nm within 5 minutes.

Result judgment

There are two methods to judge the results: the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the Nitroimidazoles concentration.

Qualitative determination

The concentration range (ng/mL) of Nitroimidazoles can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.3, and that of the sample Ⅱ is 1.0, the OD value of standard solutions is: 2.243 for 0ppb, 1.816 for 0.1ppb, 1.415 for 0.3ppb, 0.74 for 0.9ppb, 0.313 for 2.7ppb, 0.155 for 8.1ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1ppb, and that of the sample Ⅱ is 0.1 to 0.9ppb.

Quantitative determination

The mean values of the absorbance values obtained for the percentage of the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

Percentage of absorbance value = $(B / B_0) \times 100\%$

B—the average OD value of the sample or the standard solution

B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Nitroimidazoles standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Nitroimidazoles concentration in the sample.

Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

Precautions

1. The room temperature below 25 ℃ or the temperature of the reagents and the samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lots to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard solution and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the colouration solution with any color that indicates the degeneration of this solution. The detecting value of the standard solution 1(0 ppb) of less than 0.5 indicates its degeneration.

Storage: store at 2-8 ℃, not frozen.
Expiry date: 12 months; date of production is on box.