IMMUNOMART

VERSION 1.01

DEXAMETHASONE ELISA TEST KIT

MANUAL

ELISAKITS.ONLINE

By Immunomart

Dexamethasone ELISA Test Kit

Catalogue Number. IP100045

Principle

This test kit is based on the competitive enzyme immunoassay for the detection of Dexamethasone in the sample. The coupling antigen is pre-coated on the micro-well stripes. The Dexamethasone in the sample and the coupling antigen pre-coated on the micro-well stripes compete for the anti-Dexamethasone antibody. 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 Dexamethasone in it. This value is compared to the standard curve and the Dexamethasone concentration is subsequently obtained.

Technical specifications

Sensitivity: 0.1ppb
Incubation Temperature: 25℃
Incubation Time: 30min ～ 15min
Detection limit: Tissue 0.1 ppb, Feed 0.2 ppb, Milk powder 0.3 ppb, Milk 0.2 ppb, Honey 0.2 ppb, Urine 0.2 ppb
Cross-reaction rate: Dexamethasone 100%
Recovery rate: Tissue, feed 90±25%, Milk powder 85±25%, Urine, milk, honey 95±25%

Components

1	Micro-well strips	12 strips with 8 removable wells each	
2	6× standard solution (1mL each)	0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb	
3	Enzyme conjugate	7ml	red cap
4	Antibody working solution	7ml	blue cap
5	Substrate A	7ml	white cap
6	Substrate B	7ml	black cap
7	Stop solution	7ml	yellow cap
8	20× concentrated washing buffer	15ml	white cap
9	5× concentrated redissolving solution	110ml	transparent cap

Materials required but not provided

Equipments: microplate reader, homogenizer, nitrogen-drying device, vortex, centrifuge, measuring pipets, and balance(a sensibility reciprocal of 0.01 g), Incubator, printer.
Micropipettors: single-channel 20-200 μL, 100-1000 μL, and multi-channel 30～300 μl.
Reagents: Ethyl acetate, Acetonitrile (CH_3CN), 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 checked to be clean and should be re-cleaned if necessary, in order to avoid the contamination which interferes with the experimental results.

Solution preparation before sample pre-treatment

1) Acetonitrile and Ethyl acetate mixing solution: 1 part Acetonitrile + 1 part Ethyl acetate

2) Sample redissolving solution: the 5×concentrated redissolving solution is diluted with deionized water at 1:4.

Samples preparation

a) Tissue

1. Take 2.0 ± 0.05 g of the homogenized sample into a 50ml centrifuge tube; Add 2ml sample redissolving solution, then add 6ml Ethyl acetate, shake for 3min, centrifuge at above 4000 r/min at 15℃ for 10 min.
2. Take 3mL clear organic phase into a dry container, blow to dry by nitrogen or air in 50-60℃.
3. Dissolve the dry residues in 1 mL N-hexane, add 1 mL of the sample redissolving solution, mix for 30 seconds; centrifuge at above 4000 r/min at 15℃ for 10 min, remove the up-layer N-hexane phase.
4. Take 50 µL of the down-layer for analysis.

Fold of dilution of the sample: 1

b) Feed

1. Weigh 1.0±0.05g homogenized feed sample into 50ml centrifuge tube, add 6ml ethyl acetate, shake for 3 min, centrifuge at above 4000 r/min at 15 ℃ for 10 min.
2. Take 3 ml clear organic phase into a dry container, blow to dry with nitrogen or air at 50-60℃.
3. Dissolve dry residues in 1 mL N-hexane, add 1 mL of the sample redissolving solution, mix for 30 seconds, centrifuge at above 4000 r/min at 15℃ for 10 min, remove up-layer N-hexane phase.
4. Take 50 µL down-layer liquid for further analysis.

Fold of dilution of the sample: 2

c) Milk powder

1. Weigh 1.0 ± 0.05 g milk powder into a 50ml centrifuge tube, add 2ml sample redissolving solution, then add 6ml Acetonitrile and Ethyl acetate mixing solution, shake for 3min, centrifuge at above 4000 r/min at 15℃ for 10 min.
2. Take 2ml clear organic phase into a dry container, blow to dry with nitrogen or air at 50-60 ℃.
3. Dissolve dry residues in 2ml N-hexane, add 1ml sample redissolving solution, mix for 20 seconds, centrifuge at above 4000 r/min at 15℃ for 10 min, remove up-layer N-hexane phase.
4. Take 50 µL down-layer liquid for further analysis.

Fold of dilution of the sample: 3

d) Honey

1. Weigh 1.0±0.05 g honey into a 50ml centrifuge tube, add 2ml sample redissolving solution, then add 6ml Ethyl acetate, shake for 3min. Centrifuge at above 4000 r/min at 15℃ for 10 min.
2. Take 3ml clear organic phase into a dry container, blow to dry with nitrogen or air at 50-60 ℃.
3. Dissolve dry residues in 1ml N-hexane, add 1ml sample redissolving solution, mix for 30 seconds, centrifuge at above 4000 r/min at 15℃ for 10 min, remove up-layer N-hexane phase.
4. Take 50 µL down-layer liquid for further analysis.

Fold of dilution of the sample: 2

e) Urine

1. Take 1.0ml urine sample into a 5ml centrifuge tube, add 2ml Ethyl acetate, shake for 1min, centrifuge at above 4000 r/min at 15℃ for 10 min.
2. Take 1ml clear organic phase into a dry container, blow to dry with nitrogen or air at 50-60 ℃.
3. Dissolve dry residues in 1ml N-hexane, add 1ml sample redissolving solution, mix for 30 seconds, centrifuge at above 4000 r/min at 15℃ for 10 min, remove up-layer N-hexane phase.
4. Take 50 µL down-layer liquid for further analysis.

Fold of dilution of the sample: 2

f) Milk

1. Take 1.0ml milk sample into a 5ml centrifuge tube, add 2ml Acetonitrile and Ethyl acetate mixing solution, shake for 1min, centrifuge at above 4000 r/min at 15℃ for 10 min.
2. Take 1ml clear organic phase into a dry container, blow to dry with nitrogen or air at 50-60 ℃.
3. Dissolve dry residues in 1ml N-hexane, add 1ml sample redissolving solution, mix for 30 seconds, centrifuge at above 4000 r/min at 15℃ for 10 min, remove up-layer N-hexane phase.
4. Take 50 µL down-layer liquid for further analysis.

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. Take out all the necessary reagents from the kit and place at the room temperature (20-25 ℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, store at 2-8℃, not frozen.
3. Solution preparation: dilute 15 mL of the concentrated washing buffer (20 × concentrated) with the deionized water at 1:19 (1 part of 20X concentrated washing buffer + 19 parts of deionized water), or prepare as quantity needed.
4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate, record their positions.
5. Add 50 µL of the sample or standard solution to separate duplicate wells; and add enzyme conjugate, 50ul/well, then add antibody working solution, 50µl/well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25 ℃ for 30 min.
6. Pour the liquid, wash the microplate with the washing buffer at 250 µL/well for 4-5 times. Each time soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 50 µL of the substrate A solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 15 min at dark for coloration.

8. Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of microplate reader at 450 nm to determine the OD value. (recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

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 content of Dexamethasone.

Qualitative determination

The concentration range (ng/mL) can be obtained from the comparison 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, while those of the standard solutions are as the followings: 2.243 for 0 ppb, 1.816 for 0.1 ppb, 1.415for 0.3 ppb, 0.74 for 0.9 ppb, 0.313 for 2.7 ppb and 0.155 for 8.1 ppb, accordingly the concentration range of the sample Ⅰ is 2.7 to 8.1 ppb, and that of the sample Ⅱ is 0.3 to 0.9 ppb.

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 Dexamethasone 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, thus finally obtaining the Dexamethasone 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.
3. Mix every reagent and reaction mixture evenly and wash the microplate thoroughly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin;
5. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colourless color former is light sensitive, and thus they cannot be directly exposed to the light.
6. 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 lot numbers to use.

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.
8. The optimum reaction temperature is 25 ℃, and too high or too low temperatures will result in the changes in the detecting sensitivity and OD values.

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