

Operation Manual of the kit for Detection of Papaverine

(For Use in Food Inspection)

Based on the principle of solid enzyme-linked immunosorbent assay, the kits can be used to detect papaverine by direct competitive immunosorbent reactions between anti- papaverine antibody and antigen-enzyme conjugate or tested antigen, combining with catalytic color reaction of the enzyme. The kits are characterized by high sensitivity, good specificity and easy to operate and read out.

一、Composition of the reagent kit:

1. antibody-coated microplate:	48 wells
2.reagent A: sample diluent	1 vial
3.reagentB:papaverine standard solution (10 μg/kg)	1 vial
4. reagent C: antigen-enzyme conjugate	2 vial
5. reagent D: diluent for antigen-enzyme conjugate	1 vial
6. reagent E: concentrated washing solution (300*)	1 vial
7. reagent F: substrate solution A	1 vial
8. reagent G: substrate solution B	1 vial
9. reagent H: stopping solution	1 vial
10. microplate holder	1 plate

二、Preparations for test:

1. Sample treatment:

(1) Solid sample: weight 5.0g sample, add 50ml hot water then boil it for about 20 minutes, filter it. Wash the filter residue with a small amount of hot water. Mix the eluent with the filtrate and

constant-volume at 50.0ml. Add 20.0ml constant volume liquid into a separatory funnel(125ml) with 15ml hexane or petroleum ether, shake the funnel for 5 minutes, let the lower extract layer (fat free liquid) out of the funnel when it is stationary and layered. Dilute 0.1ml fat free liquid with 0.2ml reagent A, wait to determine.

(2). Liquid samples: add 20.0 ml liquid sample into a separatory funnel (125ml) with 5ml hexane or petroleum ether, shake the funnel for 5 minutes, let the lower extract layer (fat free liquid) out of the funnel when it is stationary and layered. Dilute 0.1ml fat free liquid with 0.2ml reagent A, wait to determine.

The method of diluting fat free liquid:(because of no enough reagent A, we suggest you dilute as follows)

First: mix 0.05ml fat free liquid with 0.2 ml reagent A;

Second: dilute 0.05ml the above mixed liquid with 0.25ml reagent A, wait to determine.

2. Preparation of reagents:

(1) Each vial of reagent C is added with 1.5ml reagent D, dissolved and mixed. It can be stored for use within 6 months at 2-8°C.

(2) Adding 300ml distilled water into reagent E makes up the washing solution.

(3) Let the reagent kit to be at room temperature.

(4) Wash the microplate twice with reagent E at an interval of 1 minute. Notice that the washing solution shouldn't be overflow and the microplate should be patted to be dry on a water-absorbing paper.

三 Test procedure

1. Number the microplate wells:

No. 1—3 are wells for standard control test. No. 4—12 are sample wells.

2. Inject the wells with reactants:

Prepared solutions and tested sample diluents are injected into the wells in an orderly way as indicated in the following table.

order	volume to be added	well number											
		1	2	3	4	5	6	7	8	9	10	11	12
1	50 μ l	A	B	A									
2													
3	50 μ l	D	C	C	C	C	C	C	C	C	C	C	C
4													

3. Reaction:

Incubated at 37°C for 30 minutes after the above addition of reagents.

4. Washing :

Take out the microplate and wash it five times with washing solution E at intervals of 2 minutes.

Notice that the washing solution should not be over flow and the microplate should be patted to be dry on a water-absorbing paper after washing.

5. Coloration

Add 50 μ l substrate solution A and 50 μ l substrate solution B into each well of the microplate.

Shake and then incubated at 37°C for 15 minutes for visual estimation.

6. Stopping:

Add 50 μ l stopping solution (reagent H) into each well to stop reactions for instrumental detection.

7. Detection:

Compare colors of well 1—3 .If the color of well 3 is the deepest, followed by well 2, and the

color of well 1 is nearly colorless, it demonstrates that the standard control is correct.

(1) Visual estimation: (visual before the adding of stopping solution)

Compare color of sample well with that of the well 2. If the color of the sample well is not deeper than that of the well 2, it is positive. Conversely ,it is negative. If the colors of the two wells are approximately the same, then it is necessary to detect it by instrument method or by normal international test method.

(2) Instrumental detection:

Determine value A at 450nm with the instrument. It is positive if $A_{\text{sample}} \leq A_{\text{well 2}}$, and it is negative if $A_{\text{sample}} > A_{\text{well 2}}$.

8. Result

Positive $\geq 300 \mu \text{g/kg}$

Negative $< 300 \mu \text{g/kg}$

六. Cautions

1. In order to avoiding pollution, containers and pipet tips contaminated with reagent B or reagent C must be immersed in 5% NaClO solution for one day, and then washed for another use.
2. Reagents with different lot number must not be used at the same time.

七. Exp

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