Crystal violet (CV) ELISA Kit

Technical Manual

(ELISA)

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1 Principle and Application

This kit adopts the method of indirect competitive enzyme-linked immunoassay (ELISA) to detect Crystal violet (CV) in the sample such as fish and shrimps. The kit is composed of Microtiter Plate coated with coupled antigens, HRP conjugate, antibodies, standards and other supporting reagents. During the detection, with adding standards or samples, the CV in the samples will compete with the coupled antigens to combine with anti-CV antibodies. After adding HRP conjugate, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with CV content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the CV content in the sample.

2 Technique Data

2.1 Kit Sensitivity: 0.05ppb (ng/mL)

2.2 Reactive Mode: 25°C, 30min ~ 30min ~ 15min

2.3 Detection Limits:

Sample	Detection Limits
Fish / shrimps	0.1ppb

2.4 Cross-reaction Rate:

Crystal violet 100%

Leucocrystal Violet(Oxidized) 100%

2.5 Sample Recovery Rate:

Sample	Recovery rate
Fish / shrimps	85±15%

3 Composition of the Kit

Reagent	Specification
Microtiter Plate	8wells× 12strips
High Standard (black cap)： 20ppb (The Solution is volatile and needs to be sealed)	1×1.0mL
Standard: 0ppb, 0.05ppb, 0.1ppb, 0.2ppb, 0.4ppb, 0.8ppb (black cap; all are empty bottles. You need to prepare solutions before use.)	
Antibody Solution (blue cap)	1×5.5mL
HRP Conjugate (red cap)	1×11mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Cosolvent (yellow cap)	1×6mL
Oxidant (black cap)	1×6mL
Concentrated Reconstitution Buffer (10×) (yellow cap)	1×20mL
Concentrated Wash Buffer (20×) (white cap)	1×40mL
Instruction	1
Adhesive Membrane	1
Sealed bag	1

4 Materials Required but Not Supplied

4.1 Equipment: microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (for shake and mix), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;

4.2 Micropipette: single-channel (20-200μL, 100-1000μL), and multi-channel 300μL;

4.3 Reagents: Acetonitrile (chromatographically pure), ethyl acetate, methanol.

5 Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

5.1 Notice Before Sample Processing:

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

5.2 Solution preparation:

Solution 1: Reconstitution Buffer

For example, to prepare 100 mL of Solution 1, you need to take 10 mL of Concentrated Wash Buffer (10×), then add 50mL of deionized water and 40mL of methanol, and mix thoroughly.

Solution 2: Working wash buffer

Dilute the concentrated wash buffer (20×) by a factor of 20 (Concentrated wash buffer/Deionized water= 1: 19).

5.3 Sample pretreatment steps:

5.3.1 Sample (fish and shrimps) treatment.

1) Remove the skin, bone and fat of the fish/ shrimp,

then grind the sample using a grinder.

2) Weigh 1g±0.05g of homogenized samples into a 50mL centrifuge tube, add 0.3mL of acetonitrile, then add 6mL of ethyl acetate. Shake them for above 5min to ensure that the sample is not caked.

3) Centrifuge them at 4000 rpm at room temperature for 10min. Take 3mL of supernatant to a 10mL glass test tube, add 50μL of oxidant, and shake for 2 min.

4) Add 50 mL of cosolvent to the tube (do not shake the tube), dry it using nitrogen or air at 50°C. (There should be a drop of Solution at the bottom of the tube after drying. If the sample has a high fat content, it will be visible that there are yellow sticky droplets remaining at the bottom of the tube.)

5) Add 1mL of Reconstitution Buffer (Solution 1), mix them well, and take out 50μL for test.

Dilution times of the sample:2 Detection limits: 0.1ppb

6 ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Before starting the experiment, it is necessary to prepare the standards (Prepare the low-concentration standard when it is to be used as it is unstable; The prepared standard is stable at 4°C for a month).

Add 3mL of **Solution 1** to the vial labeled as 0ppb. Add 2.88mL of **Solution 1** to the vials labeled as 0.05ppb, 0.1ppb, 0.2ppb, and 0.4ppb, respectively. Add 2.88mL of **Solution 1** to the vial labeled as 0.4ppb.

Standard 6: Pipette 120μL of the 20ppb **high standard** to the vial labeled as 0.8ppb (containing 2.88mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.8ppb.**

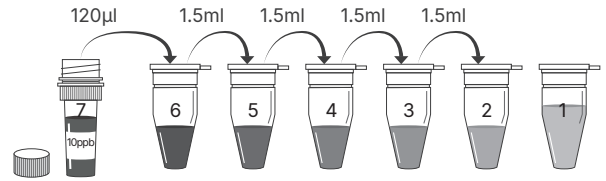
Standard 5: Pipette 1.5mL of standard 6 to the vial labeled as 0.4ppb (containing 1.5mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.4ppb.**

Standard 4: Pipette 1.5mL of standard 5 to the vial labeled as 0.2ppb (containing 1.5mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.2ppb.**

Standard 3: Pipette 1.5mL of **standard 4** to the vial labeled as 0.1ppb (containing 1.5mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.1ppb.**

Standard 2: Pipette 1.5mL of **standard 3** to the vial labeled as 0.05ppb (containing 1.5mL of **Solution 1**). Close the vial tightly, mix thoroughly, and the concentration will be **0.05ppb.**

Standard 1: The **Solution 1** can be directly used, and the concentration is **0 ppb.**



tube	7	6	5	4	3	2	1
	20ppb	0.8ppb	0.4ppb	0.2ppb	0.1ppb	0.05ppb	0ppb

Step 1: Number: Number the samples and standard corresponding to wells in order, make 2-well parallel trials for each sample and standard, and record the locations of sample wells and standard wells.

Step 2: Sample Incubation: Add 50μL of standard or sample into each numbered well, then add 50μL of antibody solution per well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C.

Step 3: Washing: Uncover the adhesive membrane carefully, remove the liquid, pipette 350μL of Working Wash Buffer (Solution 2) to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after patting dry can be punctured with a clean pipette tip).

Step 4: Enzyme Incubation: Add 100μL of HRP conjugate into each well. Then cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 30 min at 25°C.

Step 5: Washing: Same as step 3.

Step 6: Color: Add 50μL of Substrate Reagent A to each well. Then add 50μL of Substrate Reagent B per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 25°C in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

Step 7: Stop the reaction: Pipette 50μL of Stop Solution to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 8: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

7 Interpretation of result

7.1 Calculate the percentage of absorbance value

Percentage of absorbance value(%)= $\frac{A}{A0}$ ×100%

A—the average OD value of the sample or standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

7.2 Draw the standard curve and calculate

Take absorbance percentage(A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.

Draw the standard semi-log curves with X-axis and Y-axis.

Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of CV of samples.**

If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

8 Attention

8.1 Before test, the reagents and samples should be balanced to room temperature (25°C). If below 25°C, it will lead to all the standard OD value on the low side.

8.2 In the washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.

8.3 Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.

8.4 During the incubation, cover microplates with adhesive membrane to avoid light.

8.5 Do not use kits that are overdue. Do not mix reagents with those from other lots.

8.6 Substrate Reagent A/B is colorless. If not, please discard.

8.7 If absorbance value of 0ppb is below 0.5（A450nm< 0.5), it means that the reagent may be metamorphic.

8.8 Stop solution is corrosives, please avoid contact with skin.

8.9 As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.

8.10 For the mentioned sample, fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.

8.11 The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.

9 Storage conditions

The kit shall be stored at 2-8 °C. Avoid freezing.

Shelf Life: 12 months. The date of manufacture is presented in the label of the box.