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Catalase (CAT) Assay Kit

(Visible Light Method)

Cat. No: abk310 Pack： 100T/96S

1. Assay principle:

Ammonium molybdate can pause H_2O_2 decomposing reaction catalyzed by catalase (CAT) immediately, residual H_2O_2 can react with ammonium molybdate to produce a yellowish complex. It is able calculate CAT activity by measuring OD value at 405nm.

2. Reagent composition and preparation

Reagent 1: Solution 100ml ×1 bottle, can be stored at 4C for 6 months.

Reagent 2: Substrate solution 10ml ×1 bottle, can be stored at 4C for 6 months.

Reagent 3: Chromogenic agent powder× 1 bottle, can be stored at 4 C for 6 months. Add double distilled water until volume reaches 10 0 ml, dissolve sufficiently. Prepared solution can be stored at 4 C for 1 month. If there is undissolved powder at bottom, then please take supernant to use directly, it will not disturb results.

Reagent 4: Solution 10ml ×1 bottle, can be stored at 4C for 6 months.

It will freeze in cold days, then please put it in 37 C water bath until limpid before use.

3. Operation procedures:

(1) Blood serum (or plasma) CAT assay:

① **Operation table:**

	Contrast tube	Sample tube
Blood serum (or plasma) (ml)		0.1
Reagent 1 (Prewarmed at 37C) (ml)	1.0	1.0
Reagent 2 (Prewarmed at 37C) (ml)	0.1	0.1
Mix sufficiently, reacts at 37C for 1 minute accurately		
Reagent 3 (ml)	1.0	1.0
Reagent 4 (ml)	0.1	0.1
Blood serum (or plasma) (ml)	0.1	
Mix sufficiently, transfer in cuvettes of 0.5 cm light path, measure OD values of all tubes at 405 nm (adjust zero by distilled water)		

② Blood serum (or plasma) CAT activity calculation:

a. **Definition:** 1μmol H_2O_2 decomposing per ml blood serum (or plasma) per second is considered as 1 activity unit (U).

b. Formula:

$$\text{Blood serum (or plasma) CAT activity (U/ml)} = (OD_{Contrast} - OD_{Sample}) \times 271^{*} \times \frac{1}{60 \times \text{Sample volume}} \times \text{Sample dilution times before assay}$$

Note: * 271 is reciprocal value of slope.

c. Example:

Take 0. 1ml blood serum to measure CAT activity, in results, ODContrast is 0.720, ODSample is 0.675.

Calculate as follows:

$$\text{Blood serum (or plasma) CAT activity (U/ml)} = (0.720-0.675)\times 271\times\frac{1}{60\times 0.1}\times 1 = 2.03\ U/ml$$

Note: In order to operate expediently, you can do all preparations at first, label all test tubes by numbers, add 0. 1ml sample and 1ml Reagent 1, then put all test tubes in 37C water bath for 3~5 minutes. During operation, add 0. 1ml Reagent 2, count time accurately at the same time, mix sufficiently immediately, place in 37C water bath, when t= 1 min, add chromogenic agent immediately and terminate reaction, mix sufficiently. Then you can operate Tube 2, Tube 3 …contrast tube & sample tube. Contrast tube and sample tube must be measured at same time.

(2) Whole blood CAT assay:

① **1:99 hemolysate preparation:** Take 50μl whole blood, add double distilled water until volume reaches 5ml, place for 10 minutes and then you can start assay.

② Operation table:

	Blank contrast tube	Sample tube	Self contrast tube
Double distilled water (ml)	0.05		2.1
1 : 99 hemolysate (ml)		0.05	0.05
Reagent 1 (Prewarmed at 37C) (ml)	1.0	1.0	
Reagent 2 (Prewarmed at 37C) (ml)	0.1	0.1	
Mix sufficiently, reacts at 37 C for 1 minute accurately			
Reagent 3 (ml)	1.0	1.0	
Mix sufficiently, transfer in cuvettes of 0.5 cm light path, measure OD values of all tubes at 405 nm (adjust zero by double distilled water)			

③ Hemoglobin CAT activity calculation:

a. **Definition:** 1 μmol H_2O_2 decomposing per mg hemoglobin per second is considered as 1 activity unit (U).

b. **Formula:**

$$\text{Hemolysate CAT activity (U/mgHb)} = (OD_{\text{Blank contrast}} + OD_{\text{Self contrast}} - OD_{\text{Sample}}) \times \frac{271^* \times 1}{60 \times \text{Sample volume}} \div \text{Hemoglobin (mgHb/ml)}$$

Note: * 271 is reciprocal value of slope.

c. Example:

Take 0.05 ml 1:99 mouse hemolysate to measure CAT activity, in results, ODBlank contrast is 0.569, ODSelf contrast is 0. 105, ODSample is 0.516, hemoglobin content in 1:99 hemolysate is 1.324mgHb/ml.

Calculate as follows:

$$\text{Hemolysate CAT activity (U/mgHb)} = (0.569 + 0.105 - 0.516) \times 271 \times \frac{1}{60 \times 0.05} \div 1.324 = 10.78 \ U/mgHb$$

(3) Tissue homogenate CAT assay:

① **Tissue homogenate preparation:** Weigh tissue accurately, add 9 times physiological saline according to mass(g)-volume(ml) ratio of 1:9, make 10% tissue homogenate in icewater bath , centrifugate at 2500 rpm for 10 minutes. Take supernatant, dilute with physiological saline to optimal sample concentration for assay (you can read optimal sample concentration probing in appendix).

② **Operation table:**

	Contrast tube	Sample tube
Homogenate (ml)		0.05
Reagent 1 (Prewarmed at 37C) (ml)	1.0	1.0
Reagent 2 (Prewarmed at 37C) (ml)	0.1	0.1
Mix sufficiently, reacts at 37C for 1 minute accurately		
Reagent 3 (ml)	1.0	1.0
Reagent 4 (ml)	0.1	0.1
Homogenate (ml)	0.05	
Mix sufficiently, transfer in cuvettes of 0.5 cm light path, measure OD values of all tubes at 405nm (adjust zero by double distilled water)		

③ Tissue homogenate CAT activity calculation:

a. **Definition:** 1 μmol H_2O_2 decomposing per mg tissue protein per second is considered as 1 activity unit (U).

b. Formula:

$$\text{Tissue homogenate CAT activity (U/mgprot)} = (OD_{Contrast} - OD_{Sample}) \times 271^{*} \times \frac{1}{60 \times \text{Sample volume}} \div \text{Protein concentration in tissue homogenate (mgprot/ml)}$$

Note: * 271 is reciprocal value of slope.

c. Examples:

① Take 0.05 ml 0.5% rat liver tissue homogenate to measure CAT activity, in results, ODContrast is 0.671, ODSample is 0.445, protein content in 0.5% rat liver tissue homogenate is 0.48mgprot/ml

Calculate as follows:

$$\text{Tissue homogenate CAT activity (U/mgprot)} = (0.671 - 0.445) \times 271 \times \frac{1}{60 \times 0.05} \div 0.48 = 42.53 \ U/mgprot$$

② Take 0.05 ml 5% earthworm tissue homogenate to measure CAT activity, in results, ODContrast is 0.605, ODSample is 0.425, protein content in 5% earthworm tissue homogenate is 3. 1158 mgprot/ml Calculate as follows:

$$\text{Tissue homogenate CAT activity (U/mgprot)} = (0.605-0.425)\times 271\times \frac{1}{60\times 0.05}\div 3.1158 = 5.2185\ U/mgprot$$

③ Take 0.05 ml 10% rice leaf homogenate to measure CAT activity, in results, ODContrast is 0.704, ODSample is 0.332, protein content in 10% rice leaf homogenate is 3. 1303 mgprot/ml Calculate as follows:

$$\text{Tissue homogenate CAT activity (U/mgprot)} = (0.704-0.332)\times 271\times \frac{1}{60\times 0.05}\div 3.1303 = 10.7351\ U/mgprot$$

④ Take 0.05 ml 0.5% *Acipenser sinensis* liver tissue homogenate to measure CAT activity, in results, ODContrast is 0.580, ODSample is 0.426, protein content in 0.5% *Acipenser sinensis* liver tissue homogenate is 0.3063mgprot/ml Calculate as follows:

$$\text{Tissue homogenate CAT activity (U/mgprot)} = (0.580-0.503)\times 271\times \frac{1}{60\times 0.05}\div 0.3063 = 22.7058\ U/mgprot$$

Note: **(1) When you do blood serum (or plasma) CAT assay, if there is no hemolysis in samples, then you just need to take 2 random samples for contrast per batch or use double distilled water for contrast; if there is hemolysis, then you must make contrast tube for each sample.**

(2) When do tissue homogenate CAT assay, if there is no hyperlipemia, then you just need to take

2 random samples for contrast per batch or use double distilled water for contrast; if there is hyperlipemia, then you must make contrast tube for each sample.

(3) When you do whole blood CAT assay, you must make contrast tube for each sample.

Appendix: Optimal sample volume probing

1. **Sample source:** Normal mouse liver tissue,

2. **Pretreatment:**

10% homogenate preparation: Weigh tissue accurately, add 9 times physiological saline according to mass(g)- volume(ml) ratio of 1 : 9 , make 10 % tissue homogenate in icewater bath , centrifugate at 2500 rpm for 10 minutes, take supernatant for assay,

Dilute homogenate with physiological saline to different concentrations: 5% 、 2% 、 1% 、 0.5%、 0.25% 、 0. 125%

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3. Operation table:

	Contrast tube	Sample tube
Homogenate (ml)		0.05
Reagent 1 (Prewarmed at 37 C) (ml)	1.0	1.0
Reagent 2 (Prewarmed at 37 C) (ml)	0.1	0.1
Mix sufficiently, reacts at 37 C for 1 minute accurately		
Reagent 3 (ml)	1.0	1.0
Reagent 4 (ml)	0.1	0.1
Homogenate (ml)	0.05	
Mix sufficiently, transfer in cuvettes of 0.5 cm light path, measure OD values of all tubes at 405 nm (adjust zero by double distilled water)		

4. Result:

OD Contrast	0.671					
Homogenate concentration	0. 125%	0.25%	0.5%	1%	2%	5%
ODSample	0.613	0.558	0.445	0.258	0.059	0.02
Absolute OD	0.058	0. 113	0.226	0.413	0.612	0.651

5. Mouse liver tissue optimal sample concentration probing curve:

Referenced sample concentration: 0.25%~1%. In this range, enzyme cuvre appears direct proportion relatively after regression curve treatment. If sample concentration is too high or too low,

then results won't appear significant deviation after statistical treatment.