Copper-zinc superoxide dismutase(CuZn-SOD) assay kit

(Cat/No.:BC155 Size:100T/48S,50T/24S)

1. Assay Principle (Hydroxylamine Method)

Use xanthine and xanthine oxidase reaction system to produce superoxide anion radicals O_2 the latter will oxidate hydroxylamine to form nitrite, appears prunosus color under effect of chromogenic agent, its absorbance can be measured by visible range spectrophotometer. If sample to assay contains SOD, then it has a narrow spectrum depressant effect for superoxide anion radicals, as result, absorbance in sample tube will be lower than absorbance in contrast tube, SOD activity can be calculated by formula.

There are only 3 types of SOD in plant cells, they are CuZn-SOD, Mn-SOD and Fe-SOD. Fe-SOD and Mn-SOD are majority in lower plant cells while CuZn-SOD is majority in higher plant cells. Generally, Mn-SOD exists in cytoplasm and chloroplasts, Mn-SOD exists in mitochondria and Fe-SOD exist in chloroplasts of some plants. Sum of 3 types SOD is considered as total SOD (T-SOD). Mn-SOD and Fe-SOD loss acitivity in pretreated samples and CuZn-SOD activity keeps stable.

2. Composition and preparation (The kit is vaid for 1 year) (100T/48S)

Reagent No.	Reagent name	Pack	Storage
Reagent 1	Buffer stock solution	10ml×1 bottle	At 4℃ for 1 year
		(Some cystals may seed out in cold days or in fridge, please put bottle in hot water bath to dissolve crystals before use)	
	Reagent 1 working solution preparation: When use, dilute each bottle with double distilled water until volume reaches 100ml, working solution can be stored at 4℃ for 1 year.		
Reagent 2	Substrate solution	10ml×1 bottle	At 4℃ for 1 year
Reagent 3	Matrix solution	10ml×1 bottle	At 4℃ for 1 year
Reagent 4	Enzyme stock	350μl×2 vials	**At -20℃** for 6 months

	solution		
	Enzyme diluent	10ml×1 bottle	At 4℃ for 1 year
	Enzyme working solution preparation: When use, mix enzyme stock solution and enzyme diluent at ratio of 1:14, **consider solution volume according to you need. Prepared enzyme working solution can be stored at 4℃, do not freeze.**		
Reagent 5	Powder×1 vial		At 4℃ for 1 year
	Reagent 5 preparation: When use, add 75ml double distilled water at 70℃~80℃, dissolve powder before use. If water volume reduces by evaporation in heating process, then add more double distilled water to make volume reaches 75ml. Prepared solution can be stored **at 4℃ away from light for 1 year.**		
Reagent 6	Powder×1 vial		At 4℃ for 1 year
	Reagent 6 preparation: When use, add 75ml double distilled water to dissolve, prepared solution can be stored **at 4℃ away from light for 6 months**		
Chromogenic agent	**Chromogenic agent preparation:** Mix Reagent 5, Reagent 6 and glacial acetic acid at ratio of 3:3:2, prepared solution can be stored at **4℃ away from light for 3 months.** **Note:** Glacial acetic acid, analytical pure, acetic acid ≥99.5%		
Reagent 7	CuZn-SOD extraction solution A	12ml×1 bottle	At 4℃ hermetically away from light for 1 year
	CuZn-SOD extraction solution B	12ml×1 bottle	At 4℃ hermetically away from light for 1 year
Reagent 8	Homogenate medium	60ml×1 bottle	At 4℃ hermetically away from light for 1 year

3. Reagent composition and preparation (50T/24S)

Reagent No.	Reagent name	Pack	Storage
Reagent 1	Buffer stock solution	5ml×1 bottle	At 4℃ for 1 year
		(Some cystals may seed out in cold days or in fridge, please put bottle in hot water bath to dissolve crystals before use)	
	Reagent 1 working solution preparation: When use, dilute each bottle with double distilled water until volume reaches 100ml, working solution can be stored at 4℃ for 1 year.		
Reagent 2	Substrate solution	5ml×1 bottle	At 4℃ for 1 year
Reagent 3	Matrix solution	5ml×1 bottle	At 4℃ for 1 year
Reagent 4	Enzyme stock	350μl×1 vial	**At -20℃** for 6 months

	solution		
	Enzyme diluent	5ml×1 bottle	At 4℃ for 1 year
	Enzyme working solution preparation: When use, mix enzyme stock solution and enzyme diluent at ratio of 1:14, **consider solution volume according to you need. Prepared enzyme working solution can be stored at 4℃, do not freeze.**		
Reagent 5	Powder×1 vial		At 4℃ for 1 year
	Reagent 5 preparation: When use, add 37.5ml double distilled water at 70℃~80℃, dissolve powder before use. If water volume reduces by evaporation in heating process, then add more double distilled water to make volume reaches 37.5ml. Prepared solution can be stored **at 4℃ away from light for 1 year.**		
Reagent 6	Powder×1 vial		At 4℃ for 1 year
	Reagent 6 preparation: When use, add 37.5ml double distilled water to dissolve, prepared solution can be stored **at 4℃ away from light for 6 months**		
Chromogenic agent	**Chromogenic agent preparation:** Mix Reagent 5, Reagent 6 and glacial acetic acid at ratio of 3:3:2, prepared solution can be stored at **4℃ away from light for 3 months.** **Note:** Glacial acetic acid, analytical pure, acetic acid ≥99.5%		
Reagent 7	CuZn-SOD extraction solution A	6ml×1 bottle	At 4℃ hermetically away from light for 1 year
	CuZn-SOD extraction solution B	6ml×1 bottle	At 4℃ hermetically away from light for 1 year
Reagent 8	Homogenate medium	60ml×1 bottle	At 4℃ hermetically away from light for 1 year

4. Operation procedure

(1) 20% plant tissue homogenate preparation: Weigh plant tissue sample accurately (0.2~0.5g), add

4 times volume homogenate medium according to mass(g)-volume(ml) ratio of 1:4, cut tissue to small pieces, make homogenate in icewater bath. Centrifugate at 3500rpm for 10 minutes, take supernatant for assay.

(2) T-SOD optimal sample volume probing:

Take 0.1ml 20% homogenate supernatant, add 0.2ml homogenate medium (equals to 3 times dilution), mix sufficiently, take 3 samples of different volumes (10μl, 30μl, 50μl), do pre-test according to

operation table in order to determine optimal sample volume.

$$\text{Inhibition percentage}=\frac{OD_{\text{Contrast}}-OD_{\text{T-SOD sample}}}{OD_{\text{Contrast}}}\times 100\%$$

Optimal sample volume range: Curve appears direct proportion while inhibition percentage is between 15~55%. **Take the tube which inhibition percentage is between 45% to 50% as optimal sample volume.**

Optimal sample volume adjustment: If inhibition percentage is over 60% (curve appears "flat"), then you should dilute sample or reduce sample volume and try again. If inhibition percentage is lower
than 20%, then you should enlarge sample volume and try again.

**In this way, it is great helpful for scientific result analysis and t-test; if inhibition ratio percentage is higher than 60% or lower than 10%, then there are no significant differences between different
assay groups.**

(3) CuZn-SOD extraction: Transfer 0.1ml 20% homogenate supernatant in a test tube, then add 0.1ml CuZn-SOD extraction solution A and 0.1ml CuZn-SOD extraction solution B (**equals to 2 times dilution, CuZn-SOD extraction doesn't accept dilution**), vortex for 90s, place for 5 minutes, centrifugate at

3500rpm for 10 minutes. Now liquid becomes 3 layers, upperlayer is colorless limpid liquid (to assay), middle layer is protein layer, underlayer is colorless limpid liquid. Take upperlayer to assay according to operation table **(CuZn-SOD extract and T-SOD sample solution have same optimal sample volume**

(a*))

(4) Operation table (T-SOD optimal sample volume according to Appendix I):

	Contrast tube	**T-SOD sample tube**	**CuZn-SOD sample tube**
Reagent 1 working solution (ml)	1.0	1.0	1.0
Homogenate medium (ml)	**a***		
T-SOD sample solution(ml)		**a***	
CuZn-SOD extract (ml)			**a***
Reagent 2 (ml)	0.1	0.1	0.1
Reagent 3 (ml)	0.1	0.1	0.1
Reagent 4 working solution (ml)	0.1	0.1	0.1
Mix sufficiently by vortex, place in 37℃ thermostatic waterbath for 40 minutes.			
Chromogenic agent (ml)	2	2	2
Mix sufficiently, place at room temperature for 10 minutes, transfer in 1cm light path cuvettes, measure OD values at 550nm (adjust zero by double distilled water).			

Note: a* is optimal sample volume.

5. Calculation

Unit definition: Corresponding quantity of SOD that its inhibition ratio pecentage reaches to 50% per gram plant tissue in this reaction system is considered as one SOD activity unit (U)

. **Formula:**

$$\text{T-SOD activity (U/g wet tissue)} = \frac{OD_{Contrast} - OD_{T\text{-}SOD\ sample}}{OD_{Contrast}} \div 50\% \times \left[\frac{\text{Reaction solution volume (ml)}}{\text{Sample volume (ml)}}\right]^{**} \times \text{Sample pretreatment dilution times (3)} \div \text{Homogenate concentration (g wet tissue/ml)}$$

$$\text{CuZn-SOD (U/g wet tissue)} = \frac{OD_{Contrast} - OD_{T\text{-}SOD\ sample}}{OD_{Contrast}} \div 50\% \times \left[\frac{\text{Reaction solution volume (ml)}}{\text{Sample volume (ml)}}\right]^{**} \times \text{Sample pretreatment dilution times (2)} \div \text{Homogenate concentration (g wet tissue/ml)}$$

Note: * is reaction system dilution times

$$^{**}\ \text{Homogenate concentration (g wet tissue/ml)} = \frac{\text{Tissue sample weight (g)}}{\text{Added homogenate medium volume (ml)}}$$

6. Example

Take 0.25g chinese cabbage leaf sample accurately, add 1ml homogenate medium, cut sample to small pieces, use mechanical homogenizer to make 20% homogenate in icewater bath. Centrifugate at
3500rpm for 10 minutes, take supernatant. Dilute supernatant with homogenate medium to 5% homogenate (4 times dilution), divide 5% homogenate to 2 part for T-SOD assay and CuZn-SOD assay separately. Pretreat 2 parts of homogenate separately, take 0.01ml, 0.03ml, 0.05ml diluted T-SOD
sample solution to do optimal samplex volume probing, as result, 0.05ml is optimal sample volume and its inhibition percentage is 43.3%, Take 0.05ml T-SOD sample solution and CuZn-SOD sample
solution separately, do experiments according to operation table. In results, $OD_{T\text{-}SOD\ sample}$ is 0.274, $OD_{CuZn\text{-}SOD}$ sample is 0.253, $OD_{Contrast}$ is 0.482, calculate as follows:

$$\text{T-SOD activity (U/g wet tissue)} = \frac{OD_{Contrast} - OD_{T\text{-}SOD\ sample}}{OD_{Contrast}} \div 50\% \times \left[\frac{\text{Reaction solution volume (ml)}}{\text{Sample volume (ml)}}\right]^{**} \times \text{Sample pretreatment dilution times (3)} \div \text{Homogenate concentration (g wet tissue/ml)}$$

$$= \frac{0.482-0.274}{0.482} \div 50\% \times \frac{3.35}{0.05} \times 3 \div \frac{1}{4} \times 4$$

$$= 2775.63U/g\text{wet tissue}$$

$$\text{CuZn-SOD (U/g wet tissue)} = \frac{OD_{Contrast} - OD_{T\text{-}SOD\ sample}}{OD_{Contrast}} \div 50\% \times \left[\frac{\text{Reaction solution volume (ml)}}{\text{Sample volume (ml)}}\right]^{**} \times \text{Sample pretreatment dilution times (2)} \div \text{Homogenate concentration (g wet tissue/ml)}$$

$$= \frac{0.482-0.253}{0.482} \div 50\% \times \frac{3.35}{0.05} \times 2 \div \frac{1}{4} \times 4$$

$$= 2037.24U/g\text{ wet tissue}$$