Vitamin E (VE) Assay kit

(Cat/No.:BC153 Size:50T/48S)

1. Compositon &Preparation(This kit is valid for 3 months, stored at 2~8℃)

Composition		Pack 50T/48S
R1	Can be stored at 2~8℃ away from light	Powder×1 vial
R1 preparation: Dissolve in 6.5ml dehydrated alcohol, this solution can be stored away from light. This powder is quite hard to dissolve, so it's better to prepare solution 3~4 hours before use. Please make sure it dissolves completely before use.		
R2	Can be stored at 2~8℃ away from light	Powder×1 vial
R2 stock solution preparation: Dissolve in 25ml dehydrated alcohol, stock solution can be stored away from light. **R2 working solution preparation**: Dilute R2 stock solution 10 times with dehydrated alcohol. Working solution can be stored at 2~8℃ for less than 2 days.		
R3	Solution, can be stored at 2~8℃	5ml×1 bottle
R4	Tissue homogenate medium, can be stored at 2~8℃	100ml×1bottle
R5	1mg/ml standard stock solution, can be stored at 2~8℃	0.6ml×1vial
12μg/ml V_E standard working solution preparation: Take 0.12ml 1mg/ml, add dehydrated alcohol until volume reaches to 10ml, 12μg/ml V_E standard working solution is prepared.		

2. Introduction

This kit can be used to measure V_E content in animal blood serum (or plasma), animal tissues & plants.

3. Assay significance

Vitamin E (VE) is a natural liposoluble antioxidant, it exists in membrane structures (such as cell membrane, mitochondrion, microsome,etc.), lipid droplets in adipocytes, circular lipoproteins.

VE is no only mundificant for singlet oxygen (O2) and superoxide anion radical (O2- ·), but also blocking agent for lipid peroxidation. Various cellular level experiments declare VE co-

operates with SoGSHPX to protect cells from lipid peroxidation damage. VE can combine with protein thionic radical to recover protein hydrosulfide group's function. VE can also cleanse LOO to avoid protein hydrosulfide group loss indirectly. Protein hydrosulfide group is an important component to preservate cell survival, so VE is first defense line of antioxidation in vivo. When VE is in shortage, organism is easy to be attacked by radicals and appears various pathological states such as senile cataract, senile nanism, etc.

4. Assay principle (Colorimetric Method)

VE can reduce Fe3+ to Fe2+, in some conditions, Fe2+ can react with phenanthrolin to produce pink compound. Using colorimetry, you can check VE content from standard curve, you can also calculate VE content by formula.

5. Operation procedures

（1）Blood serum (or plasma) V_E assay:

① V_E normal heptane extraction in blood serum (or plasma):

	Blank tube	Standard tube	Sample tube
Blood serum (or plasma) (ml)			0.1
Distilled water (ml)	0.4	0.3	0.3
12μg/ml VE standard (ml)		0.1	
Dehydrated alcohol (ml)	0.6	0.6	0.6
Mix sufficiently by vortex for 20 seconds (protein sedimentation)			
Normal heptane (ml)	1.2	1.2	1.2
Mix by vortex (extract sufficiently) for 1 minute, then centrifugate at 3000~4000 rpm for 5~10 minutes. Solution in test tube becomes 3 layers: upper layer is VE normal heptane extract, middle layer is water and alcohol, underlayer is protein sediment.			

② **Chromogenic reaction:**

	Blank tube	Standard tube	Sample tube
VE normal heptane extract (ml)	0.8	0.8	0.8
R1 (ml)	0.1	0.1	0.1
R2 (ml)	0.05	0.05	0.05
Mix sufficiently, record time immediately, place quescently for 5 minutes accurately			
R3 (ml)	0.05	0.05	0.05
Mix sufficiently for about 10 seconds			
Dehydrated alcohol (ml)	1	1	1
Mix sufficiently, place for 2 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 533nm (adjust zero by dehydrated alcohol).			

③ **Calculation:**

a. Formula:

$$V_E\ \text{content}\ (\mu g/ml) = \frac{OD_U - OD_B}{OD_S - OD_B} \times C_S \times N$$

OD_U----------Sample tube OD value OD_S----------Standard tube OD value

OD_B----------Blank tube OD value C_S----------Standard concentration (12μg/ml)

N----------Sample dilution times before assay

b. Example:

Take 0. 1ml blood serum, measure VE content according to operation procedure, in results, ODB is 0.016, ODS is 0.077, OBU is 0.061, calculate as follows:

$$\text{Blood serum}\ V_E\ \text{content}\ (\mu g/ml) = \frac{OD_U - OD_B}{OD_S - OD_B} \times C_S \times N$$

(2) Tissue V_E assay:

① **Tissue homogenate preparation:** Weigh tissue accurately, add R4 (homogenate medium) to make homogenate, centrifugate at 3000rpm for 10 minutes, take supernatant to assay.

② V_E normal heptane extraction in tissue homogenate:

	Blank tube	Standard tube	Sample tube
10% homogenate supernatant (ml)			0.4
Distilled water (ml)	0.7	0.6	0.3
12μg/ml VE standard (ml)		0.1	
Dehydrated alcohol (ml)	0.6	0.6	0.6
Mix sufficiently by vortex for 20 seconds (protein sedimentation)			
Normal heptane (ml)	1.2	1.2	1.2
Mix by vortex (extract sufficiently) for 1 minute, then centrifugate at 3000~4000 rpm for 5~10 minutes. Solution in test tube becomes 3 layers: upper layer is VE normal heptane extract, middle layer is water and alcohol, underlayer is protein sediment.			

③ **Chromogenic reaction:**

	Blank tube	Standard tube	Sample tube
VE normal heptane extract (ml)	0.8	0.8	0.8
R1 (ml)	0.1	0.1	0.1
R2 (ml)	0.05	0.05	0.05
Mix sufficiently, record time immediately, place quescently for 5 minutes accurately			
R3 (ml)	0.05	0.05	0.05
Mix sufficiently for about 10 seconds			
Dehydrated alcohol (ml)	1	1	1
Mix sufficiently, place for 2 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 533nm (adjust zero by dehydrated alcohol).			

④ **Calculation:**

a. Formula:

$$\frac{V_E \text{ content}}{(\mu g/g \text{ wet tissue})} = \frac{OD_U - OD_B}{OD_S - OD_B} \times C_S \div 4 \div \frac{\text{Wet tissue weight}}{(g/ml)}$$

b. Example:

Take 0.4ml 10% normal liver homogenate, measure VE content according to operation procedure, in results, ODB is 0.023, ODS is 0.080, OBU is 0.046, calculate as follows:

$$\frac{\text{VE content}}{(\mu g/g\,\text{wet tissue})} = \frac{0.046-0.023}{0.080-0.023} \times 12\mu g/ml \div 4 \div 0.1 g/ml$$

$$= 12.105 \mu g/g\,\text{wet tissue}$$

6. Announcements

(1) Please wash test tubes with boiled soapsuds or detergents at first, then wash them with tap water, rinse them with distilled water at last.

(2) R2 working solution is better to be prepared & used in same day, how much you need, how much you should prepare.

(3) Chromogenic reaction time length should be 5 minutes accurately.

(4) VE extraction time lenghth should not be less than 1 minute.

(5) This method is microscaled, so when you change tip, 1st sucked solution should be discarded, then every sample or reagent should be added vertically, do not add on tube surface.

(6) When you suck normal heptane extract, please be careful enough to avoid sucking middle layer (water & alcohol layer), or it will disturb OD value.

(7) Test tubes for chromogenic reaction should be dryed completely.

(8) This kit can be used for scientific research & laboratory only.