GENLISA® Potassium Assay

Cat No: KBCA2016

I. Determination Principle:

In alkaline medium, potassium ions in serum samples treated with protein precipitants react with NA-TPB to produce turbid and stable suspensions. The turbidity is proportional to the potassium ion concentration in the sample.

II. Reagent Composition and preparation:

96T	Components	Specification	save
Reagent 1	Nail Liquid	20ml×1 bottle	at 4ºC for 6 months
	Liquid B	2.5ml×1bottle	at 4ºC for 6 months
	Preparation of protein precipitation agent: Prepare according to the ratio of Liquid A:Liquid B = 8:1 and prepare it before use.		
Reagent 2	NA-TPB working fluid	25ml×1 bottle	at 4ºC for 6 months
Reagent 3	0.8mmol /l potassium standard solution	1ml×1 tube	at 4ºC for 6 months
	Preparation of 0.4mmol/L potassium standard solution: Mix 0.8mmol /L potassium standard solution and deionized water in a ratio of 1:1.		

III. Sample Requirements:

1. Collect and process samples according to routine inspection requirements, samples can be serum (plasma), tissue homogenate, cells and culture supernatant.

2. The sample can be stable for 3 to 4 days at 2 to 8ºC and for several months at below -20ºC.

IV. Operation Steps:

1. **Sample pretreatment:**
   Serum (plasma) sample : Take 20 ul of serum (plasma) and add 180ul of protein precipitant , centrifuge at 3500 rpm for 5 minutes , and take 50 ul of supernatant for measurement.

   Tissue samples : Accurately weigh the tissue , add 9 times **deionized water** at a ratio of weight (g): volume (mL) = 1:9, homogenize in an ice water bath, centrifuge at 2500 rpm for 10 minutes, take 20ul of the supernatant and add 180ul of protein precipitant , centrifuge at 3500 rpm for 5 minutes, and take 50ul of the supernatant for measurement.

Operation Table:

	Blank Well	Standard Well	Sample Well
Deionized water (ul)	50		
0.4mmol /l potassium standard solution (ul)		50	
Supernatant of sample to be tested (ul)			50
Working solution (ul)	200	200	200
Mix well, let stand for 5 minutes, and measure the absorbance of each well using a microplate reader at 440nm.			

Note: Before the experiment, you can read and record the OD value of the empty plate using a microplate reader. To ensure the accuracy of the experiment.

V. Calculation Formula:

1. Serum Calculation formula:

$$\text{Serum (Plasma) Potassium concentration (nmol/l)} = \frac{A_{measurement} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \times 10$$

2. Organization calculation formula :

$$\text{Potassium Content in Tissue (mmol/gprot)} = \frac{A_{measurement} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times 10 \div Cpr$$

$$\text{Potassium Content in Tissue (mmol/gtissue)} = \frac{A_{measurement} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times 10 \div \frac{W}{V_{extract}}$$

C standard: standard concentration, 0.4mmol/l;
N: dilution multiple of the sample before testing (after adding the precipitant and before mixing with the working solution);
10: dilution factor when the sample is added with protein precipitant;
Cpr: protein concentration of tissue homogenate, gprot/l (prot refers to protein);
W: tissue weight, g;
V extract : the total volume of extract added during tissue homogenization, L.

VI. Technical Parameters:

Linear Range	0.01 ~ 0.8 mmol/l	Intra-batch variation	≤4%
Batch difference	≤6%	Kit shelf life	6 months

Note:

1. Red blood cells contain high concentrations of potassium ions, so hemolyzed samples are not acceptable.
2. Ammonia, mercury and chlorine may interfere with the determination of potassium.
3. When preparing tissue homogenate, it is best to use deionized water as the homogenate medium and potassium contamination should be avoided.
4. This kit can be used on fully automatic / semi - automatic biochemical analyzers.

5. Determine the potassium ion content in tissues or cells. It is recommended to simultaneously determine the total protein concentration.
6. This kit is for scientific research only.

Appendix I: Preparation of Potassium Standard Curve

1. Pre-treatment:

Use deionized water to gradient dilute the 0.8mmol/l potassium standard solution into different concentrations : 0.025mmol/l , 0.05mmol/l , 0.1 mmol/l , 0.2 mmol/l , and 0.4 mmol/l .

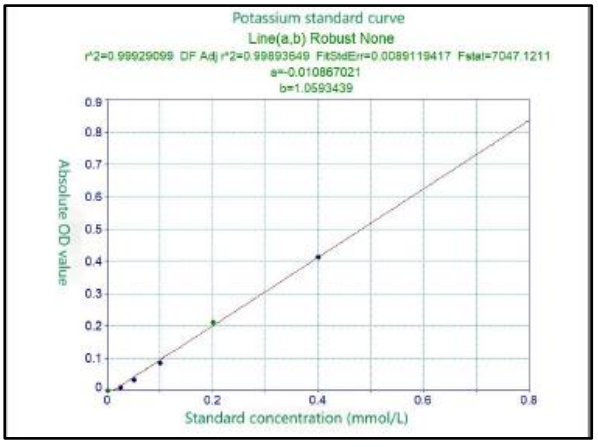
2. Operation table:

	Blank Well	Standard Well
Deionized water (ul)	50	
potassium standard solutions of different concentrations (ul)		50
Working solution (ul)	200	200
Mix well, let stand for 5 minutes, and measure the absorbance of each well using a microplate reader at 450nm.		

3. Measurement results:

Standard concentration (mmol/l)	Determination of OD value	Absolute OD value
0	0.0697	0
0.025	0.0802	0.0105
0.05	0.1042	0.0345
0.1	0.1564	0.0867
0.2	0.2818	0.2121
0.4	0.483	0.4133
0.8	0.905	0.8353

4. Drawing:



Appendix II: Determination of potassium in serum samples

1. Pre-treatment:

Take 20 ul of serum (plasma), add 180 ul of protein precipitant, centrifuge at 3500 rpm for 5 minutes, and take 50 ul of supernatant for measurement.

2. Operation table:

	Blank Well	Standard Well	Sample Well
Deionized water (ul)	50		
0.4mmol /l potassium standard solution (ul)		50	
Supernatant of sample to be tested (ul)			50
Working solution (ul)	200	200	200
Mix well, let stand for 5 minutes, and measure the absorbance of each well using a microplate reader at 450nm .			

Note: Before the experiment, the 96- well plate can be read and recorded with an ELISA reader. To ensure the accuracy of the experiment.

3. Calculation formula :

$$\text{Serum (Plasma) Potassium concentration (nmol/l)} = \frac{A_{measurement} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N \times 10$$

C standard: standard concentration, 0.4mmol/l;
N: dilution multiple of the sample before testing (after adding the precipitant and before mixing with the working solution);
10: dilution factor of the sample when protein precipitant is added.

4. Calculation example:

Example 1: Take 20 ul of rat serum (plasma) and measure according to the instructions: the OD value of the blank well is 0.0697, the OD value of the standard well is 0.4830, and the OD value of the test well is 0.5763. The calculation result is:

$$\text{Serum (Plasma) Potassium concentration (mmol/l)} = \frac{0.5763 - 0.0697}{0.4830 - 0.0697} \times 0.4 \times 10 \times 1$$

$$= 4.9034 \text{ mmol/l}$$

Example 2: Take 20 ul of fish serum (plasma) and measure according to the instructions: the OD value of the blank well is 0.0697, the OD value of the standard well is 0.4830, and the OD value of the test well is 0.2621. The calculation result is:

$$\text{Serum (Plasma) Potassium concentration (mmol/l)} = \frac{0.2621 - 0.0697}{0.4830 - 0.0697} \times 0.4 \times 10 \times 1$$

$$= 1.8619 \text{ mmol/l}$$

Appendix III : Potassium Determination in Tissue Samples

1. Pre-treatment:
Accurately weigh the tissue, add 9 times deionized water at a ratio of weight (g): volume (mL) = 1:9, homogenize in an ice water bath, centrifuge at 2500 rpm for 10 minutes, take 20 ul of the

supernatant, add 180 ul of protein precipitant, centrifuge at 3500 rpm for 5 minutes, and take 50 ul of the supernatant for measurement.

	Blank Well	Standard Well	Sample Well
Deionized water (ul)	50		
0.4mmol /l potassium standard solution (ul)		50	
Supernatant of sample to be tested (ul)			50
Working solution (ul)	200	200	200
Mix well, let stand for 5 minutes, and measure the absorbance of each well using a microplate reader at 450nm .			

Note: Before the experiment, the 96-well plate can be read and recorded with an ELISA reader. To ensure the accuracy of the experiment.

4. Calculation formula:

$$\text{Potassium Content in Tissue (mmol/gprot)} = \frac{A_{measurement} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times 10 \div Cpr$$

$$\text{Potassium Content in Tissue (mmol/gtissue)} = \frac{A_{measurement} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times 10 \div \frac{W}{V_{extract}}$$

C standard : standard concentration, 0.4mmol/l;
10: dilution factor of the sample when the protein precipitant is added;
Cpr: protein concentration of tissue homogenate, gprot/l (prot refers to protein);
W: tissue weight, g;
V: total volume of extract added during tissue homogenization, L.

4. Calculation example:

Example 1: Take 20 ul of 10% homogenate supernatant of a certain tissue and measure it according to the instructions: the OD value of the blank well is 0.0697, the OD value of the standard well is 0.4830, the OD value of the test well is 0.6699, and the protein concentration of the 10% squid gill homogenate supernatant is 4.5851; the calculation result is:

$$\text{Potassium Content in Tissue (mmol/gprot)} = \frac{0.6699 - 0.0697}{0.4830 - 0.0697} \times 0.4 \times 10 \div 4.5851$$

$$= 1.2670 \text{ mmol/gprot}$$

Example 2: Take 20 ul of 10% brain tissue homogenate supernatant and measure according to the instructions: the OD value of the blank well is 0.0697, the OD value of the standard well is 0.4830, the OD value of the test well is 0.4350, and the protein concentration of the 10% rabbit brain tissue homogenate supernatant is 3.7909; the calculation result is:

$$\text{Potassium Content in Tissue (mmol/gprot)} = \frac{0.4350 - 0.0697}{0.4830 - 0.0697} \times 0.4 \times 10 \div 3.7909$$

$$= 0.9326 \text{ mmol/gprot}$$

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