PRODUCT INFORMATION & MANUAL

TRACP/PAP/ACP5 Activity Assay Kit (Colorimetric)

NBP3-24536

For research use only.
Not for diagnostic or therapeutic procedures.

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TRACP/PAP/ACP5 Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24536

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 9.22 U/L

Detection range: 9.22-100 U/L

Average intra-assay CV (%): 2

Average inter-assay CV (%): 2.3

Average recovery rate (%): 101

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure TRACP/PAP/ACP5 activity in serum, plasma, urine, animal tissue and cell samples.

▲Detection principle

The chromogenic agent can be catalyzed by acid phosphatase to produce p-nitrophenol in acidic condition. P-nitrophenol has a maximum absorption at 405 nm. The activity of TRACP/PAP/ACP5 can be calculated by measuring the amount of produced p-nitrophenol. The activity of acid phosphatase being detected in the presence of tartric acid is regarded as TRACP/PAP/ACP5 activity.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	24 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 3	Tartaric Acid Solution	1.4 mL × 2 vials	-20°C, 12 months
Reagent 4	Standard Substance	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	15 mL × 1 vial	-20°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (410-415 nm, optimum wavelength: 405 nm), Incubator (37°C)

Reagents:

Normal saline (0.9% NaCl)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

Reagent 2 working solution and the reaction working solution are easily to be decomposed in light. Avoid light during use.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:

   Dissolve a vial of reagent 2 with 0.5 mL double distilled water. Preserve it on the ice box with shading light for use and the prepared solution can be stored at -20°C with shading light for 2 days.
3. Preparation of reaction working solution:

   Mix the reagent 1 and reagent 2 working solution at a ratio of 17:1. Preserve it on the ice box with shading light for use and the prepared solution should be used up within 6 hours.
4. Preparation of 10 mmol/L standard solution:

   Dissolve a vial of reagent 4 with 1 mL of double distilled water. Mix fully for use and the prepared solution can be stored at -20°C with shading light for 2 days.
5. Preparation of 1 mmol/L standard solution:

   Mix the reagent 1 and 10 mmol/L standard solution at a ratio of 9:1. Prepare the fresh needed amount before use and the prepared solution should be stored with shading light for use.

▲ Sample preparation

1. Serum (plasma) and urine sample: Detect directly.
2. Tissue sample:

   Weigh the tissue accurately and add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 12000 g for 10 min at 4°C, then take the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Collect the 1×10^6 cells, add 200 μL normal saline (0.9% NaCl). Homogenize the cells sample with homogenizer on ice. Centrifuge the homogenized cells at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (9.22-100 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Goat plasma	1
Human urine	1
Porcine serum	1
Rat plasma	1
10% Mouse liver tissue homogenate	5-8
10% Mouse kidney tissue homogenate	3-5
10% Mouse lung tissue homogenate	2-5
10% Mouse brain tissue homogenate	3-5
1×10^6 HL-60 cells	1
1×10^6 293T cells	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note:A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 1 mmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1.0 mmol/L.Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	200
B	0.2	40	160
C	0.3	60	140
D	0.4	80	120
E	0.6	120	80
F	0.8	160	40
G	0.9	180	20
H	1.0	200	0

2. The measurement of samples

1) Standard well: Add 20 μL of standard solution with different concentrations into the corresponding wells.

   Sample well: Add 20 μL of sample into sample well.

   Control well: Add 20 μL of sample into control well.

2) Add 120 μL of reaction working solution into standard well and sample well. Add 120 μL of reagent 1 into control well.

3) Add 20 μL of reagent 3 into each well.

4) Mix fully with microplate reader for 3 s, incubate at 37°C for 10 min.

5) Add 100 μL of reagent 5 into each well and mix fully with microplate reader for 3 s, stand at room temperature for 2 min.

6) Measure the OD values of each well at 405 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well	Control well
Different concentrations standard solution (μL)	20	--	--
Sample (μL)	--	20	20
Reaction working solution (μL)	120	120	--
Reagent 1 (μL)	--	--	120
Reagent 3 (μL)	20	20	20
Mix fully with microplate reader for 3 s, incubate at 37°C for 10 min.			
Reagent 5 (μL)	100	100	100
Add 100 μL of reagent 5 into each well and mix fully with microplate reader for 3 s, stand at room temperature for 2 min. Measure the OD values of each well at 405 nm with microplate reader.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. For tissue and cell sample:

Definition: The amount of TRAP in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol p-nitrophenol at 37°C is defined as 1 unit.

$$\text{TRAP activity (U/gprot)} = (\Delta A_{405} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

2. For serum, plasma, urine sample:

Definition: The amount of TRAP in 1 L serum, plasma or urine sample per 1 minute that hydrolyze the substrate to produce 1 μmol p-nitrophenol at 37°C is defined as 1 unit.

$$\text{TRAP activity (U/L)} = (\Delta A_{405} - b) \div a \div T \times 1000 \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{405}: $OD_{Sample} - OD_{control}$.

T: The time of reaction, 10 min.

C_{pr}: The concentration of protein in sample, gprot/L.

1000: 1 mmol/L=1000 μmol/L.

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

Take 20 μL of 10% Mouse liver tissue homogenate which dilute for 5 times in normal saline (0.9% NaCl) and carry the assay according to the operation table.The results are as follows:

standard curve: y = 0.8674 x - 0.003, the average OD value of the control is 0.108, the average OD value of the sample is 0.334, $\Delta A_{405} = OD_{Sample} - OD_{control} = 0.0334 - 0.108 = 0.226$, the concentration of protein in sample is 6.77 gprot/L, and the calculation result is:

TRAP activity (U/gprot) = (0.226 + 0.003) ÷ 0.8674 ÷ 10 × 1000 ÷ 6.77 × 5

= 19.5 U/gprot