COSMO BIO CO., LTD.

L-Glutamate Assay Kit YAMASA NEO

Cat. No. YMS-80128

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【I】Features of the kit

This product has the following features for the measurement of L-glutamic acid.

- This product includes ready-to-use solution reagents.
- Only a small amount (only 10 mL) is required for measurement.
- The measurement range is wide (10-1,500 mg/L).
- Pretreatment for samples containing ascorbic acid is not required even for samples containing ascorbic acid at 1,000 mg/L.

【II】Measurement principles

R1 enzyme reagent solution is added to remove ascorbic acid (vitamin C) from samples by the action of ascorbic acid oxidase. Subsequently, R2 enzyme reagent solution is added to oxidize L-glutamic acid by the action of L-glutamic acid oxidase, generating hydrogen peroxide (1). Using hydrogen peroxide, purple color is generated through oxidative condensation between TOOS and 4-aminoantipyrine by the action of peroxidase (2). L-glutamic acid concentrations in the samples are determined based on the violet absorbance (555 nm).

【III】Product comparison table

The present product is compared with the conventional product.

Items	**L-Glutamic KIT YAMASA NEO**		Yamasa L-Glutamic acid measurement kit II	
Components	R1 enzyme reagent solution	**30 mL**	50 mM Good's buffer (pH 7.1)	60 mL
	R2 enzyme reagent solution	**30 mL**	Enzyme reagent (lyophilized)	
	L-Glutamic acid standard solution (250 mg/L)	**0.5 mL**	L-glutamic acid standard solution (100 mg/L)	1.5 mL
Amount of sample	**0.01 mL**		0.06 mL	
Measurement range	**10 ～ 1500 mg/L**		10 ～ 500 mg/L	
Samples containing	**No pre treatment required (no effect up to 1,000 mg/L ascorbic acid)**		Pretreatment required (about 30% decrease in measure values at 100 mg/L ascorbic acid	
Number of tests	**66**			
Storage	**4℃**			
Shelf life	**12 months after the date of manufacture**			

【IV】Comparison of measured values

Various samples were measured using the present and conventional products. The measured values of this product were 94 -106% of the conventional product.

Samples	L-glutamic acid concentration (mg/L)		Present product/ Conventional product
	L-Glutamic KIT YAMASA NEO (Present product)	Yamasa L-Glutamic acid measurement kit II (Conventional product)	
Grace's medium (10% FBS)	700	747	94%
Tomato juice	2032	2072	98%
Soy sauce	10701	10850	99%
Commercial soup stock solution	22327	22673	98%
Sausage solution	200	189	106%

Notes

- After extraction and removal of insoluble matters from solids, samples are appropriately diluted for measurement.
- The indicated concentrations are calculated from the measured values and the dilution rates.
- The above concentrations are our results, which do not reflect general L-glutamic acid concentrations.

Effects of ascorbic acid

To prepare samples, ascorbic acid was added to L-glutamic acid solutions at 0, 100, and 1,000 mg/L. The samples were measured using the present and conventional products.
Using the present product, no effects were observed up to 1,000 mg/L ascorbic acid.

【V】Measuring methods

1. Add 10 µL each of sample, standard solution, and purified water to tubes.
2. Add 450 µL each of R1 enzyme reagent solution to the tubes, followed by mixing.
3. Allow the tubes to stand at 20-30° C for 20 minutes. For samples for which ascorbic acid needs not be removed, skip (3) and proceed to (4).
4. Add 450 µL each of R2 enzyme reagent solution to the tubes, followed by mixing. Since the sample colors of dark samples may affect absorbance, prepare a test tube containing 10 µL of sample and 900 µL of purified water as a sample dye solution.
5. Allow the tubes to stand at 20-30° C for 20 minutes, followed by absorbance measurement at 555 nm using purified water as a control.

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	Test tubes			
	Samples	Standard solution	Purified water	Sample dye
Samples	10 µL	—	—	10 µL
Standard solution	—	10 µL	—	—
Purified water	—	—	10 µL	900 µL
R1 enzyme reagent solution	450 µL	450 µL	450 µL	—
R2 enzyme reagent solution	450 µL	450 µL	450 µL	—
Absorbance at 555 nm	**A**	**S**	**R**	**B**

【VI】Calculation of concentrations

The concentrations of L-glutamic acid in samples are calculated using the following formula:

L-glutamic acid (mg/L) = (A-B-R) ÷ (S-R) × 250 × dilution rate

※ A,B,R and S stand for the absorbance as listed above.

Notes

- Dilute liquid foods, such as soy sauce, with purified water to adjust L-glutamic acid concentrations to 10-1,500 mg/L (e.g., 100-200-fold dilution for soy sauce).
- Cut solid foods, such as cheese and sausage, into pieces. Mix these pieces with 10-20 times the amount of purified water or phosphate buffer. Cool and filter the mixture. Dilute the filtrates 2-5 fold with purified water for sample preparation. For turbid samples, repeat filtration and centrifugation. Protein removal is not needed.
- Medium can be measured without dilution. Samples with concentrations above the measurement range need to be diluted with purified water.

【VII】References

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