

Microplate Assay Kit for Glucose / Lactate / Glutamate / Glutamine



[Number of Assays]	100 assays each
[Analyte]	D-Glucose, L-Lactate L-Glutamate, L-Glutamine
[Storage Temperature]	2 - 8°C
[Expiration Date]	Indicated individually

[Contents]
D-Glucose assay reagents
Glucose Reagent A: 10 mL
Glucose Reagent B: 10 mL
D-Glucose standard solution (10 mmol/L) : 1.5 mL
L-Lactate assay reagents
Lactate Reagent A: 10 mL
Lactate Reagent B: 10 mL
L-Lactate standard solution (10 mmol/L) : 1.5 mL
L-Glutamate assay reagents
Glutamate Reagent A: 10 mL
Glutamate Reagent B: 10 mL
L-Glutamate standard solution (10 mmol/L) : 1.5 mL
L-Glutamine assay reagents
Glutamine Reagent A: 10 mL
Glutamine Reagent B: 10 mL
Six Reservoirs
Five Microplates (96 wells)

[Precautions]

- Store the reagents at 2 - 8°C.
- Bring the reagents close to room temperature (20-30°C) before use.
- More accurate measurements can be obtained by strictly controlling the reaction time to 10 minutes.
- The recommended measurement wavelength is 555 nm, but measurements can also be performed in the range of 540 to 570 nm.
- Phenol red in the sample does not affect the assay at concentrations up to 15 mg/L.

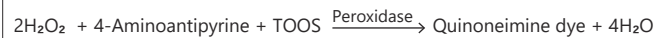
[Notes on L-Glutamine Assay]

- For L-glutamine assays, use the L-glutamate standard solution (10 mmol/L) provided with the L-glutamate assay reagents.
- The one-step measurement with L-glutamine assay reagents yields the total concentration of L-glutamine and L-glutamate. Subtract the L-glutamate concentration from the measured value to determine the L-glutamine concentration.
- When measuring L-glutamine alone (without performing L-glutamate measurement simultaneously), the protocol differs. Please refer to the instructions provided with the kit.

[Principle]

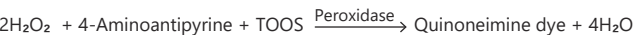
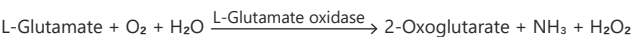
D-Glucose Assay

The enzymes in Reagents A and B catalyze the stoichiometric conversion of D-glucose into a colored product.



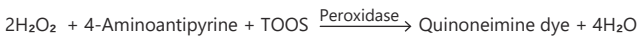
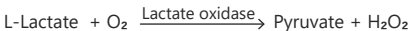
L-Glutamate Assay

The enzymes in Reagents A and B catalyze the stoichiometric conversion of L-glutamate into a colored product.



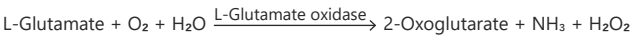
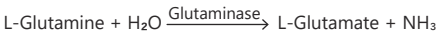
L-Lactate Assay

The enzymes in Reagents A and B catalyze the stoichiometric conversion of L-lactate into a colored product.



L-Glutamine Assay

The enzymes in Reagents A and B catalyze the stoichiometric conversion of L-glutamine and L-glutamate into a colored product.



[Sample solution]

D-Glucose / L-Lactate / L-Glutamate Assay

Sample solutions should be prepared to contain D-glucose, L-lactate or L-glutamate at a concentration of 0.05 to 2.5 mmol/L.

L-Glutamine Assay

Sample solutions should be prepared so that a total concentration of L-glutamine and L-glutamate is 0.05 to 2.5 mmol/L.

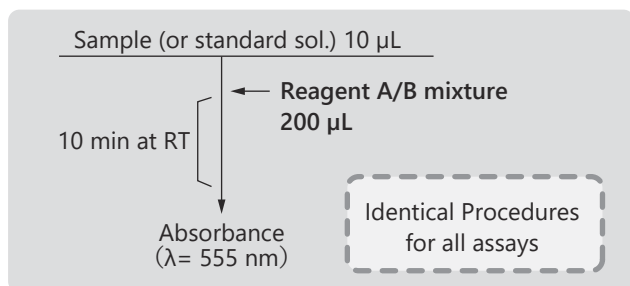
[Standard solution]

D-Glucose / L-Lactate / L-Glutamate / L-Glutamine Assay

Dilute the standard solution (10 mmol/L) provided with each assay kit before use. Note that the L-glutamate standard solution should be used for L-glutamine assay.

1. Dilute 100 μ L of the 10 mmol/L standard solution with 300 μ L of purified water to prepare a 2.5 mmol/L standard solution.
2. Dilute 100 μ L of the 10 mmol/L standard solution with 400 μ L of purified water to prepare a 2 mmol/L standard solution.
3. Dilute 75 μ L of the 10 mmol/L standard solution with 425 μ L of purified water to prepare a 1.5 mmol/L standard solution.
4. Serially dilute the 2 mmol/L standard solution 2-fold with purified water to prepare standard solutions of 1, 0.5, 0.25 and 0.125 mmol/L.
5. Use purified water for 0 mmol/L standard solution.

[Procedure] Applies to all assays



1. Mix the required amounts of Reagent A and Reagent B in a 1:1 ratio. (100 μ L of each reagent is required per well.)
*Do not store the mixture; use it on the day of preparation.

[Example of Reagent Preparation Volumes]

	24 well	48 well	96 well
Reagent A	2.6 mL	5 mL	10 mL
Reagent B	2.6 mL	5 mL	10 mL

2. Pipette 10 μ L of standard solutions or samples to each well.
3. Add 200 μ L of the reagent mixture to each well and incubate at room temperature for 10 minutes.
4. Read the absorbances at 555 nm using a plate reader.

[Reference]

[Concentration Calculation]

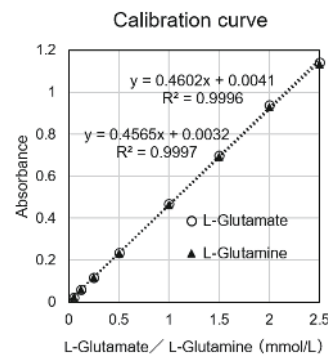
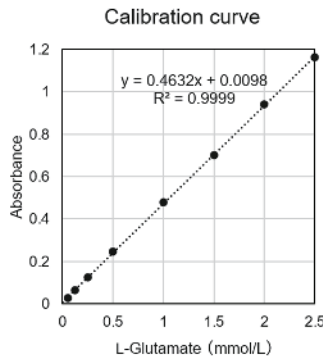
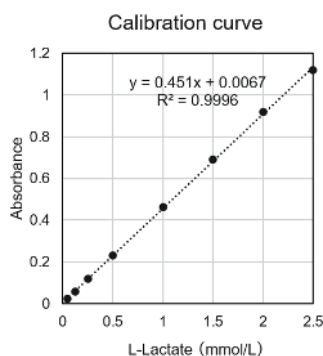
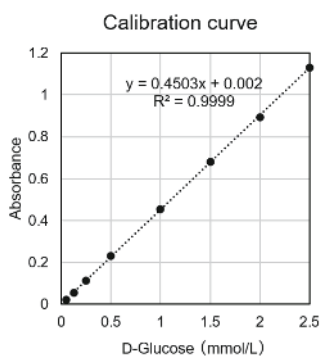
D-Glucose / L-Lactate / L-Glutamate Assay

1. Generate a calibration curve from the concentrations and absorbance values of the standard solutions.
2. Calculate the analyte concentration in the samples using the calibration curve.

L-Glutamine Assay

The one-step measurement with L-glutamine assay reagents yields the total concentration of L-glutamine and L-glutamate. To determine the L-glutamine concentration, subtract the L-glutamate concentration from the measured value.

1. Generate a calibration curve from the concentrations and absorbance values of the L-glutamate standard solutions. Calculate the total concentration of L-glutamine and L-glutamate (i) in the samples using the calibration curve.
2. To determine the L-glutamine concentration, subtract the simultaneously determined L-glutamate concentration from the total concentration (i).



Spiked recovery rate (%)

Medium Analyte	DMEM	RPMI	DMEM+FBS	RPMI+FBS
D-Glc	95.9	98.1	95.5	95.9
L-Lac	95.7	97.2	92.8	91.4
L-Glu	100.3	100.3	98.2	96.1
L-Gln	99.6	98.4	95.0	94.8

DMEM : Dulbecco's Modified Eagle's Medium
RPMI : RPMI-1640
FBS : Fetal Bovine Serum (10% final concentration)

The calibration curve generated with L-glutamate standard solutions is equivalent to that generated with L-glutamine standard solutions.



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