

Microplate Assay Kit for Glucose / Lactate / Glutamine



[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-Glutamine assay reagents	
Glutamine Reagent A:	10 mL
Glutamine Reagent B:	10 mL
L-Glutamate standard solution (10 mmol/L) :	1.5 mL
Four Reservoirs	
Five Microplates (96 wells)	
[Number of Assays]	100 assays each
[Analyte]	D-Glucose, L-Lactate, L-Glutamine
[Storage Temperature]	2 - 8°C
[Expiration Date]	Indicated individually

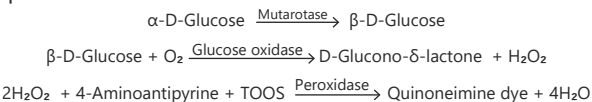
[Precautions]

- **Note the assay procedure for L-glutamine differs from that for other components.**
- 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.
- For L-glutamine assay, coexisting L-glutamate should be 5 mmol/L or less.

[Principle]

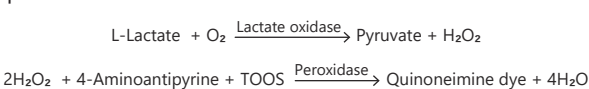
D-Glucose Assay

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



L-Lactate Assay

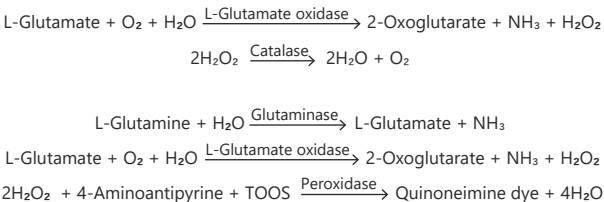
The enzymes in Reagents A and B catalyze the stoichiometric conversion of L-lactate into a colored product.



L-Glutamine Assay

Step1: The enzymes in Reagent A eliminate the effects of L-glutamate coexisting in the sample.

Step2: The enzymes in Reagent B catalyze the stoichiometric conversion of L-glutamine into a colored product.



[Sample solution]

D-Glucose / L-Lactate Assay

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

L-Glutamine Assay

Sample solutions should be prepared to contain L-glutamine at a concentration of 0.05 to 2.5 mmol/L, while ensuring that the coexisting L-glutamate concentration is less than 5 mmol/L.

[Standard solution]

D-Glucose / L-Lactate / 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]

D-Glucose / L-Lactate

The procedures for D-glucose assay and L-lactate assay are identical.

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

L-Glutamine Assay

IMPORTANT!

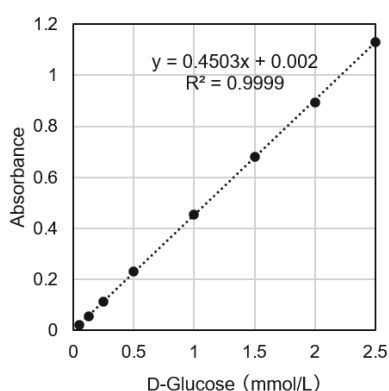
Note: Assay procedures differ between standard solutions and samples.

For L-glutamine assays, the L-glutamate standard solution is used.

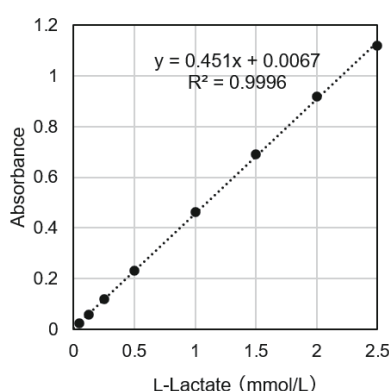
1. Pipette 10 μ L of L-glutamate standard solutions or samples to each well.
2. Add 100 μ L of Reagent B to the standard solution wells and 100 μ L of Reagent A to the sample wells, then incubate at room temperature for 10 minutes.
3. Add 100 μ L of Reagent A to the standard solution wells and 100 μ L of Reagent B to the sample wells, then incubate at room temperature for 10 minutes. Read the absorbances at 555 nm using a plate reader.
4. Generate a calibration curve from the concentrations and absorbance values of the standard solutions. Calculate the analyte concentration in the samples using the calibration curve.

[Reference]

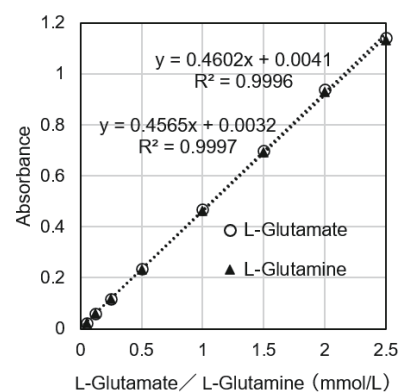
Calibration curve



Calibration curve



Calibration curve



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-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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