Cell Count Normalization Kit

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/C544.pdf

General Information

In case quantitating an enzyme activity and a metabolite in metabolic systems such as glycolysis and citric acid cycle, corrections of measured values by the number of cells are necessary to obtain exact quantitative values (see Fig. 1). Cell Count Normalization Kit employs a nucleic acid staining dye, Hoechst 33342 which binds to nuclear DNA to emit blue fluorescence. By measuring this blue fluorescence, correction of the measured value can easily be carried out in simple steps whereas the visual cell counting method requires cumbersome procedure. Moreover, unlike the correction by protein or ATP amount, the kit requires no lysis procedure. In addition, Quenching Buffer included in the kit enables a direct measuring of fluorescence signal without any background.

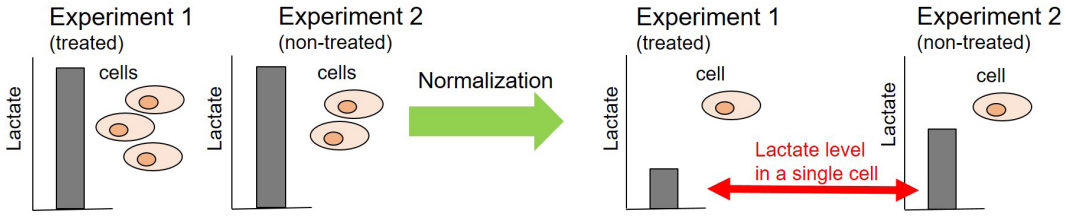


Figure 1 Necessity of normalization

Kit Contents

	200 tests	1000 tests
Staining Solution	50 µL×1	250 µL×1
Dilution Buffer	10 mL×4	100 mL×2
Quenching Buffer	10 mL×2	100 mL×1

Storage Condition

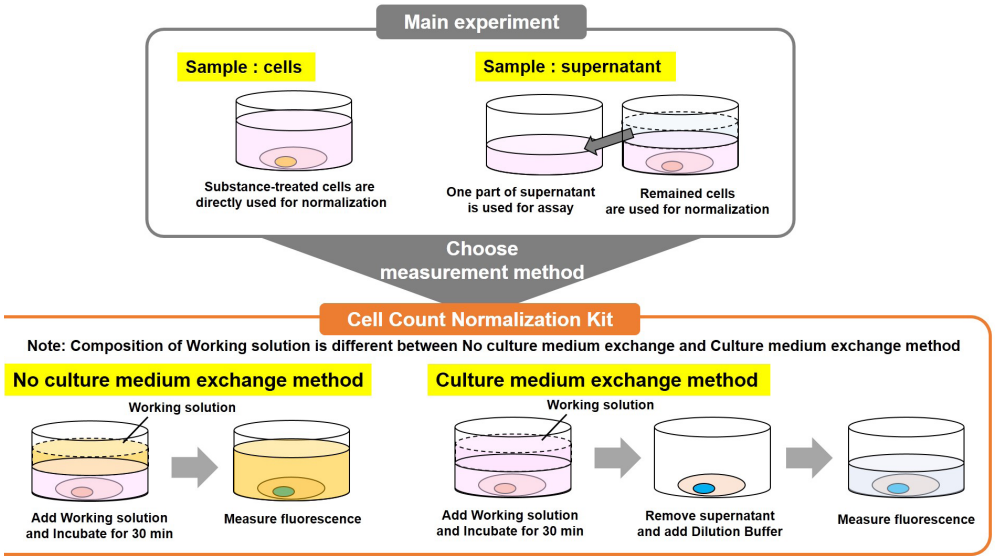
Store at 0-5 °C

Required Equipment and Materials

Microplate reader(Ex: 350 nm, Em: 461 nm)
96-well black microplate (clear bottom)
Incubator(37 °C, 5%CO_2)
Microtube(only for suspension cell)

Normalization method selection guide

Refer to the following cartoons to choose a suitable experimental procedure.



1. No culture medium exchange method
Suitable for easily detached cells because this method does not require any washing steps.
〈Procedure〉
1) Addition of Working solution
2) Incubation (30 min)
3) Reading (fluorescence)
*Please use a plate reader that can excite and read from the bottom.

2. Culture medium exchange method
Both top and bottom measurements are possible.
〈Procedure〉
1) Addition of Working solution
2) Incubation (30 min)
3) Medium replacement
4) Reading (fluorescence)

Precautions

- In order to confirm a linearity between the cell number and fluorescence signal intensity, create a calibration curve before the assay.
- Following cell numbers are recommended in case using a 96-well microplate.
  Adherent cells (HeLa cells): 1000 to 10000 cells/well, Suspension cells (Jurkat cells): 5000 to 60000 cells/well
- In case measuring a reagent blank, follow the experimental procedure of either No culture medium exchange method or Culture medium exchange method using wells without cells.
- In case using a fluorescent probe for the main experiment, simultaneous measurement are not recommended. Please make a normalization experiment after the main experiment.

Preparation of Solutions

Preparation of Working solution

1. No culture medium exchange method
   Make a 500-fold dilution of Staining Solution with Quenching Buffer
   *Use the Working solution within a day.
   *In case using 96-well microplate, 100 µL/well of Working solution is needed for each well.
2. Culture medium exchange method
   Make a 500-fold dilution of Staining Solution with Dilution Buffer
   *Use the Working solution within a day.
   *In case using 96-well microplate, 100 µL/well of Working solution is needed for each well.

General Protocol

No culture medium exchange method (only for adherent cells)

1. Seed cells and incubate an appropriate time to allow cells adhere.
2. Add 100 µL of Working solution to each well.
3. Incubate the cells at 37 °C for 30 minutes in a 5% CO_2 incubator.
4. Measure fluorescence by using a microplate reader.(bottom exciting, bottom reading, Ex: 350 nm, Em: 461 nm)

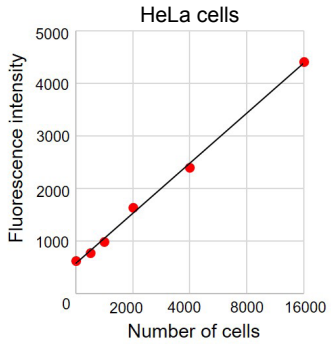


Figure 2 Calibration curve created by No culture medium exchange

Culture medium exchange method

<Adherent cells>

1. Seed cells and incubate an appropriate time to allow cells adhere.
2. Add 100 µL of Working solution to each well.
3. Incubate the cells at 37 °C for 30 minutes in a 5% CO_2 incubator.
4. Discard supernatant and add 100 µL of Dilution Buffer to each well.
5. Measure fluorescence by using a microplate reader.(Ex: 350 nm, Em: 461 nm)

<Suspension cells>

1. Seed cells.
2. Add 100 µL of Working solution to each well.
3. Incubate the cells at 37 °C for 30 minutes in a 5% CO_2 incubator.
4. Collect the cells to a microtube and centrifuge at 300×g for 5 minutes.

*If you have a centrifugation system applicable for microplates, skip the step 4 and go to the step 5.

5. Discard the supernatant then add 100µL of Dilution Buffer.
6. Measure fluorescence by using a microplate reader.(Ex: 350 nm, Em: 461 nm)

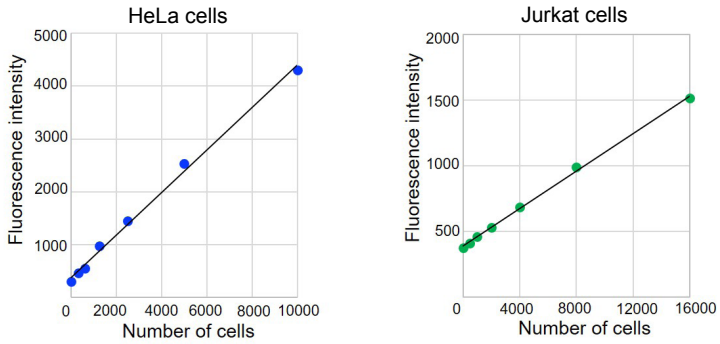


Figure 3 Calibration curves created by Culture medium exchange method

Experimental example

Evaluation of glycolysis inhibition by using 2-deoxy-D-glucose(2-DG) (Combination use with Lactate Assay Kit-WST[L256])

1. HeLa cells (5000 cells/well) were seeded on a 96 well black microplate and cultured at 37 °C overnight in a 5% CO_2 incubator
2. The supernatant was aspirated and 100 µL of 2-DG solution was added to each well. The plate was incubated 37 °C overnight in a 5% CO_2 incubator.
3. Lactate in supernatant was assayed using Lactate Assay Kit-WST by following its technical manual.
4. Assayed lactate amount (mmol/L) was normalized using Cell Count Normalization Kit (Medium exchange method).

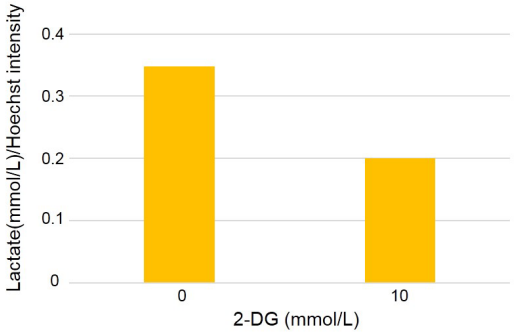


Figure 4 Amount of lactate assayed using L256 (after normalization)

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