Data Sheet

Codex EnerCount™ Cell Growth Assay Kit

Description

The EnerCount cell growth kit is a simple and sensitive assay that measures ATP levels in cultured cells, as well as in solution. The assay produces a stable luciferase-generated luminescence signal that can be recorded in commonly used luminometer instruments.

Since ATP supplies energy to all aspects of biological processes in cells, metabolically active living cells maintain robust and steady levels of ATP production. Measurement of total ATP changes has thus been used as a reliable indicator of cyto-toxicity, and cell proliferation. The EnerCount assay can be used to gauge cell growth by measuring proportional changes in total cell ATP. This is a homogenous assay, which can be performed on multi-well plates by adding the reagents directly to cell growth medium in both adherent and suspension cell cultures. This makes it ideal for automated high-throughput screening. This assay has been optimized to accurately measure cell number over a broad dynamic range (10^2 - $2x10^5$ cells) in 96-well plate format. The EnerCount assay accurately monitors cell growth over time periods from 24 – 120 h. The EnerCount assay also accurately measures ATP in solution from 0.1 to 1000 µM.

Features

- **Greater linear range:** The assay generates a linear signal while measuring 100 to 200,000 cells. It is particularly suitable for determining the proliferation of fast-growing cancer cells in typical 3-5 day growth assays.
- **Accurate:** Enercount growth curves correlate with those generated by traditional cell number counts.
- **Robust:** Excellent signal to noise (basal) ratio. Stable assay signal.
- **Easy to use:** Homogenous assay, add-and-read assay, amenable to HTS format.

Applications

- Cell proliferation
- Cell toxicity
- ATP measurement

Product Information

Catalog Number:	Components
CB-80551-010	**Assay Solution I, 5 ml** **Assay Solution II, 5 ml** **Component A, 50 µl** **Component B, 30 µl**
CB-80551-100	**Assay Solution I, 50 ml** **Assay Solution II, 50 ml** **Component A, 500 µl** **Component B, 300 µl**
CB-80551-999	**Assay Solution I, 10 X 50 ml** **Assay Solution II, 10X 50 ml** **Component A, 10 X 500 µl** **Component B, 10 X 300 µl**

Storage

Assay Solution I	Store at -20˚C
Assay Solution II	Store at -20˚C
Component A	Store at -20˚C
Component B	Store at -20˚C

The kit is stable for 6 months at -20ºC or lower temperature.

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ASSAY PROTOCOL

Preparation of Working Reagent

1. Warm the Assay Solution I and II at 37ºC until completely thawed. Prepare **1X Intermediate Solution** by mixing Solution I and II at 1:1 ratio. Keep **1X Intermediate Solution** at room temperature (~22 ºC). Assay Solution I and II can be repeatedly frozen and thawed without loss of activity. **1X Intermediate Solution** should be prepared freshly every time.
2. Thaw components A & B at room temperature. Keep Components A and B on ice. ***Note: Do not thaw Component A& B at 37˚C.***
3. Transfer 50 µl of Component A and 30 µl of Component B into 10 ml of **1X Intermediate Solution** to make **Working Reagent**. The amount of **Working Reagent** prepared can be modified to suit experimental design. Unused Components A, B, and Assay Solution I and II can be refrozen (at -20 ˚C) for future experiments.
4. Mix thoroughly by inversion and vortexing for 30 sec. The reconstituted **Working Reagent** at room temperature should be used within 1 h, or it can be kept at 4ºC for no more than 4 h. In general, only the freshly prepared **Working Reagent** is recommended for the experimental work.

Measurement of ATP in Growing Cells

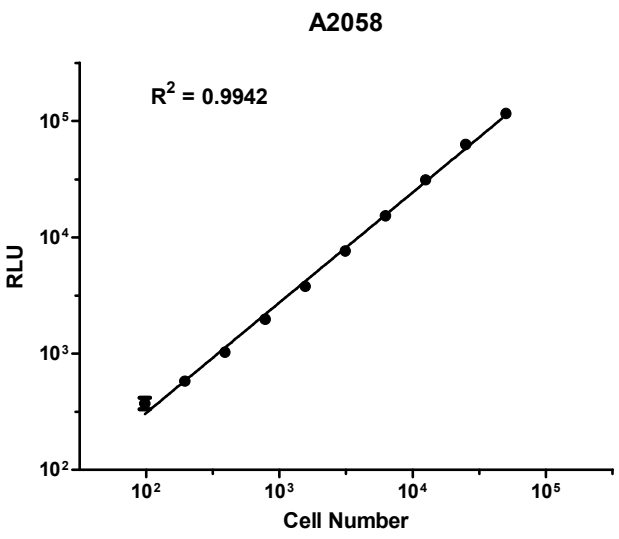
1. Grow cells on opaque white 96 well plates appropriate for luciferase signal measurement.
2. To generate assay signal, add an equal volume of **Working Reagent** to culture media overlaying cells.
3. Incubate the plate at room temperature (~22 ºC) for 60 min.
4. Read in a luminescence plate reader and record the data.

Generating an ATP standard curve

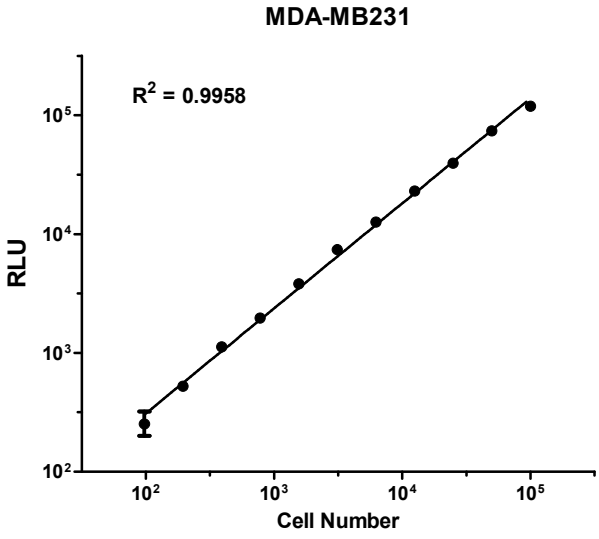
1. Dilute ATP in 1X HBSS. Transfer a fixed amount of ATP solution into the wells of a microtiter plate (e.g.,80 µl for a 96-well plate, and 20 µl for a 384-well plate).
2. Add an equal volume of **Working Reagent** into each well. Incubate the plate at room temperature (~22 ºC) for 60 min.
3. Read in a luminescence plate reader and record the data.

Data:

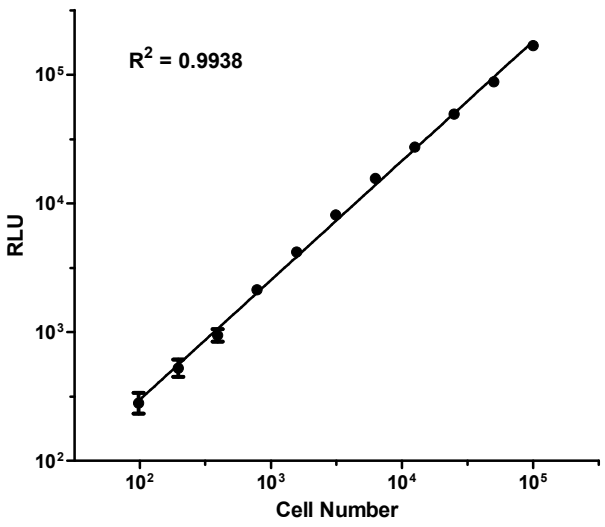
A)



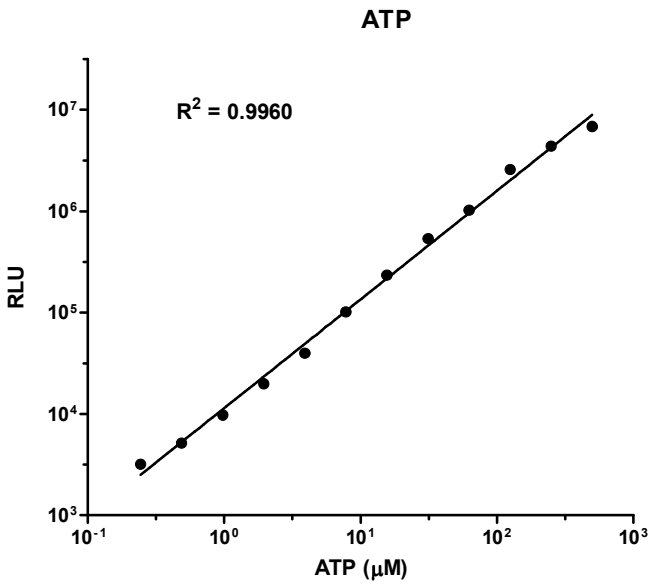
B)



C)



D)



Different numbers of A) A2058, B) MDA-MB231, C) BT549 cells were plated to a 96-well plate. **Working Reagent** (equal to cell growth media volume) from **Codex** kit was added into each well. Data was recorded 60 min later. **B)** A 1:2 serial dilution of ATP was prepared in HBSS. ATP solution (50 μl) was transferred into each well of a 96-well plate. The same volume of **Working Reagent** from the **Codex** kit was added to each well. Data was recorded 60 min later.

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