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Product Application

Automated RealTime-Glo™ MT Cell Viability Assay on the CyBio FeliX from Analytik Jena

Use the CyBio FeliX flexible pipetting platform to set up compound effect testing with the RealTime-Glo™ MT Cell Viability Assay.

Kit: RealTime-Glo™ MT Cell Viability Assay (Cat.# G9712)

Analyses: Viability (luminescence)

Sample Type: Cultured cells

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM431, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- RealTime-Glo™ MT Cell Viability Assay (Cat.# G9712)
- Test compound
- Test compound diluent
- Cells, cell medium, serum, antibiotics, (d)PBS, etc.
- CyBio FeliX Basic Unit with Enclosure (Analytik Jena, Cat.# OL5015-24-100)
- CyBio FeliX Head R 96/1000 µL (Analytik Jena, Cat.# OL3316-14-950)
- 8-Channel Adapter; Head R 96 (Analytik Jena, Cat.# OL3317-14-330)
- TipRack 96/1000 µL (Analytik Jena, Cat.# OL3317-11-140)
- Support; 97 mm height (Analytik Jena, Cat.# OL3317-11-105)
- Support; 37 mm height (Analytik Jena, Cat.# OL331-11-120)
- Waste Box (Analytik Jena, Cat.# 844-00430-0)
- Luminometer Microplate Reader (e.g. GloMax® Discover Microplate Reader (Cat.# GM3000))

Plastic Consumables Used per run:

- 2 x 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plates (Cat.# AS9307)
- 1 x 1000µl CyBio TipRack 96/1000 µl, PCR-certified (Analytik Jena, Cat.# OL3811-25-539N)
- 1 x CyBio RoboTipTray 96/1000 µL, PCR certified, pre-sterilized, filter (Analytik Jena, Cat.# OL3810-25-879)
- 1 x 96-well Solid White Flat Bottom Polystyrene TC-treated Microplates, with Lid, Sterile (Corning®, Cat.# 3917)

CyBio FeliX Method: RealTime-Glo_v1.0.bms

Method Run Time: 17 minutes

Preprocessing Time: about 45 minutes

Protocol:

The pre-processing steps described below include setting up a "Reagent Plate" and "Compound Serial Dilution Plate" and preparing cells at the appropriate initial density (to be determined by the user according to instructions from TM431 Section 4) with the RealTime-Glo™ reagents. Plate layouts are represented in Figure 1, and the instrument and deck setup are illustrated in Figure 2. Details of the automated method can be found in the next section "Method details".

1. Pre-processing:
    - Add 550µl per well of (d)PBS to a 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate column 2, rows A to H (Figure 1; Reagents Plate).
    - Prepare 4ml of culture medium containing 2x compound diluent and add 620µl per well to a 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate column 3, rows B to G (Figure 1; Reagents Plate).
    - Prepare 500µl of medium containing compound at 2x $C_{dilution\ 1}$ and add 80µl per well to a 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate column 2, rows B to G (Figure 1; Compound Serial Dilution Plate).
    - Prepare 2x RealTime-Glo™ medium: for example, add 8µl of 1,000X NanoLuc® Enzyme and 8µl of 1,000X MT Cell Viability Substrate to 4ml of culture medium for a final concentration of 2X each.
    - Add 50µl per well of 2x RealTime-Glo™ medium to a 96-well assay plate column 11, rows B to G, No-cell control wells (Figure 1; Assay Plate).
    - Resuspend the appropriate number of cells into 2x RealTime-Glo™ medium, and add 570µl per well of this cell suspension to 6 wells of a 2ml Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate column 1, rows B to G (Figure 1; Reagents Plate).

2. Load and run the CyBio FeliX method "RealTime-Glo_v1.0.bms" and follow the on-screen instructions including the deck set-up illustrated in Figure 2.

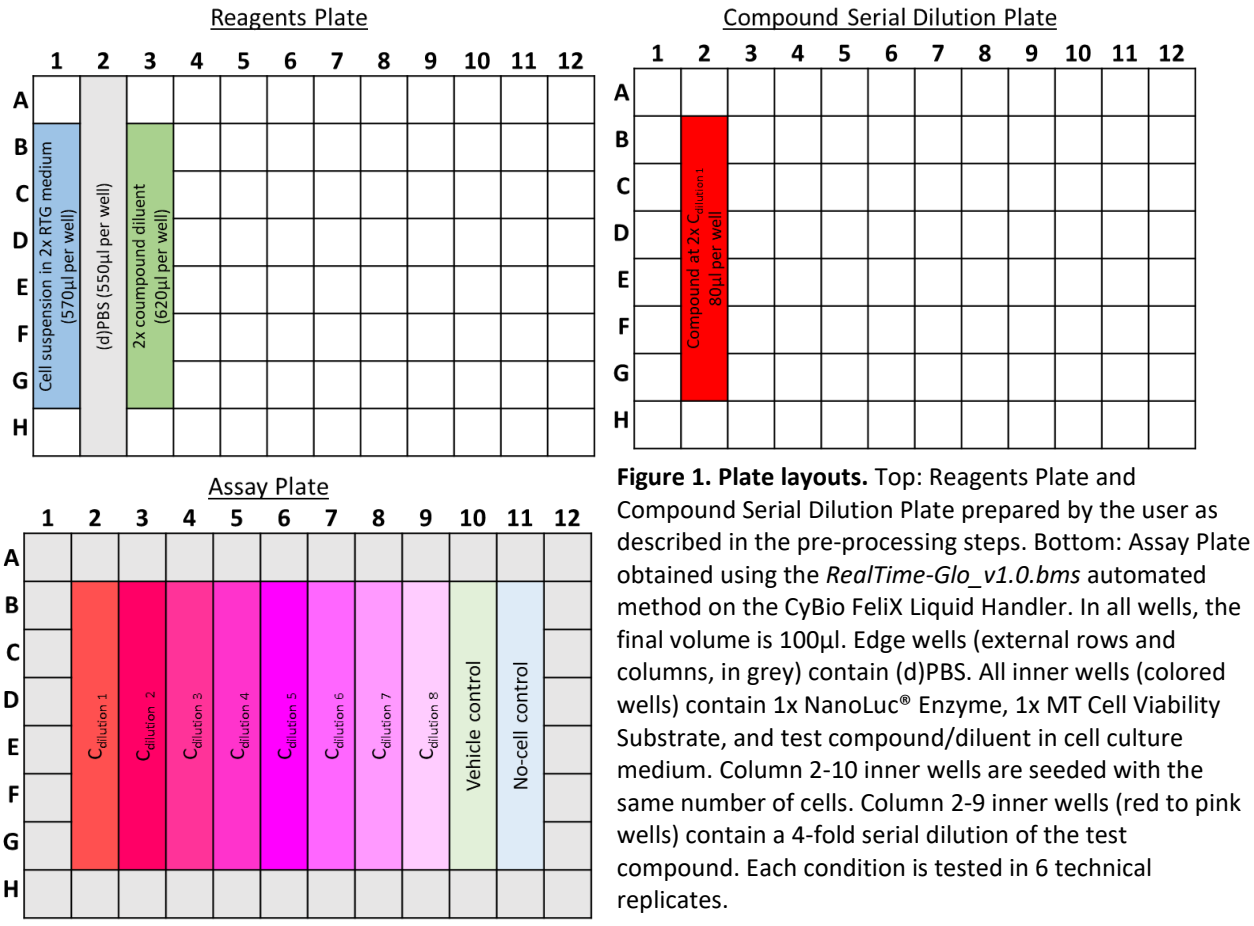
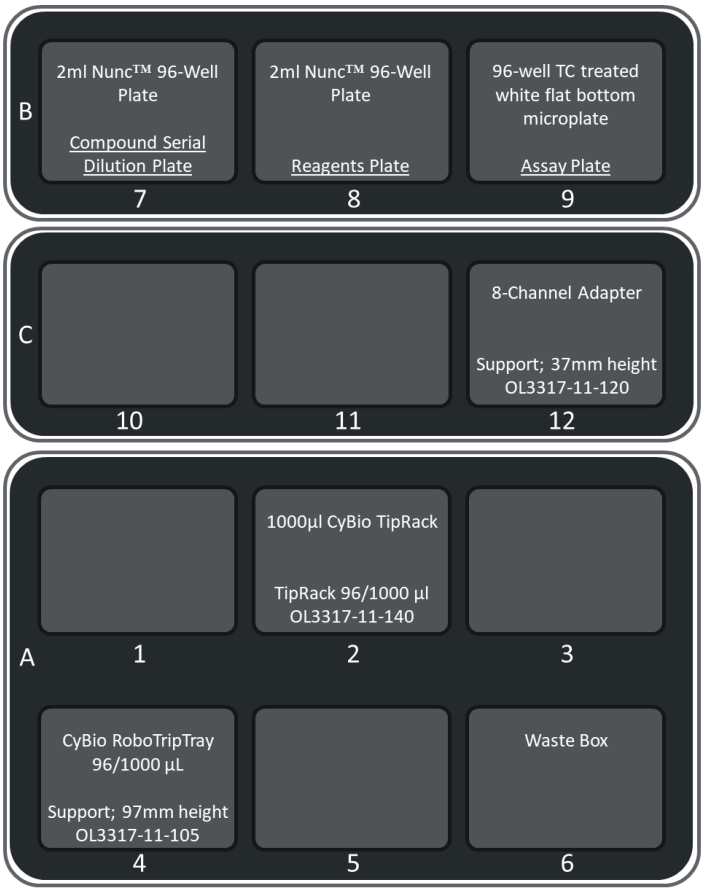


Figure 1. Plate layouts. Top: Reagents Plate and Compound Serial Dilution Plate prepared by the user as described in the pre-processing steps. Bottom: Assay Plate obtained using the *RealTime-Glo_v1.0.bms* automated method on the CyBio FeliX Liquid Handler. In all wells, the final volume is 100µl. Edge wells (external rows and columns, in grey) contain (d)PBS. All inner wells (colored wells) contain 1x NanoLuc® Enzyme, 1x MT Cell Viability Substrate, and test compound/diluent in cell culture medium. Column 2-10 inner wells are seeded with the same number of cells. Column 2-9 inner wells (red to pink wells) contain a 4-fold serial dilution of the test compound. Each condition is tested in 6 technical replicates.



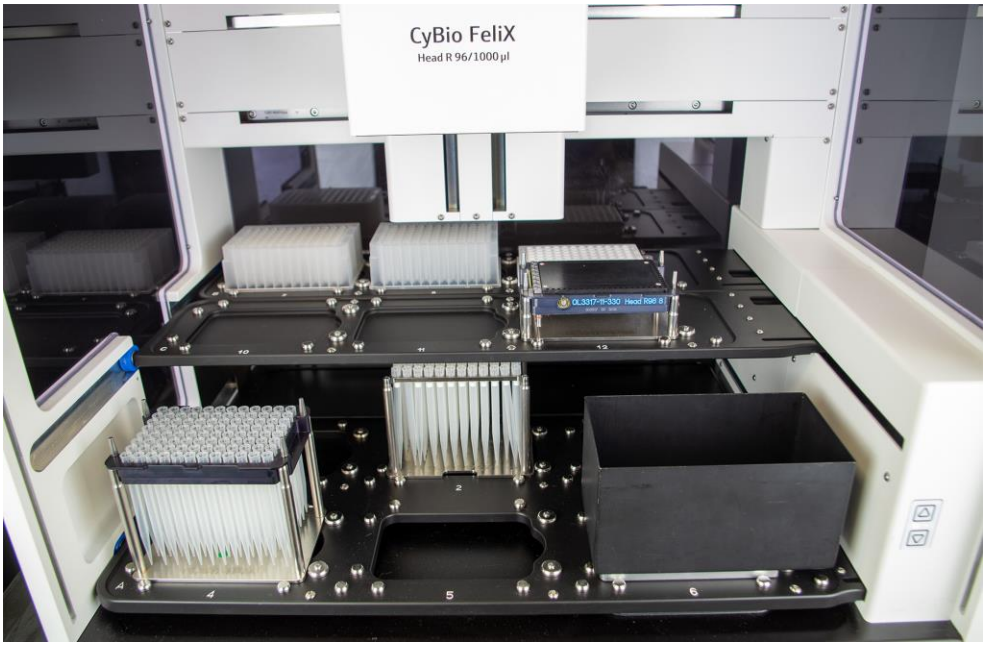


Figure 2. Deck setup. Schematic representation (top) and picture (bottom) of the deck setup. In the schematic representation, underlined are the designations for plates described in the "Method details" section. Note: The Assay Plate lid must be removed.

Method details:

This automated method enables users to set up the RealTime-Glo™ MT Cell Viability Assay on the CyBio FeliX Liquid Handler in a continuous-read format (reagent addition at cell plating) for a single cell type and a 4-fold serial dilution of a test compound (8 different concentrations), according to the plate layout presented in Figure 1. The assay plate also includes a vehicle control (compound diluent) and a No-cell control (for luminescence background subtraction).

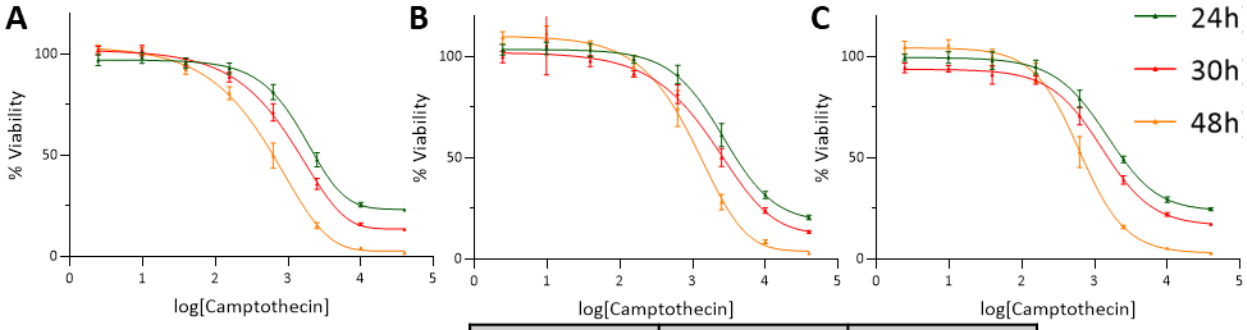
Automated method

1. Cells are tip-mixed and 50µl of the cell suspension is added to the 96-well Assay Plate (columns 2-10, rows B-G).
2. 60µl of medium containing compound diluent is added to the Compound Serial Dilution Plate (columns 3-9, rows B-G).
3. 60µl of medium containing compound diluent is added to the 96-well Assay Plate (columns 10-11, rows B-G).
4. 20µl of test compound at concentration 2x $C_{dilution\ 1}$ is transferred from wells B-G column 2 of the Compound Serial Dilution Plate into wells B-G of the next column (column 3). Compound dilution is thoroughly tip mixed. Wells B-G column 3 now contains the test compound at concentration 2x $C_{dilution\ 2}$.
5. Step 4 is repeated across the Compound Serial Dilution Plate until the 8-point dilution series is completed (i.e. 2x $C_{dilution\ 8}$ in wells B-G column 9).
6. (d)PBS is aspirated from the Reagents Plates and 100µl per well is dispensed into the outer 36 wells of the Assay Plate.
7. 50µl of test compound dilutions is aspirated from the Compound Serial Dilution Plate and dispensed into the Assay Plate using the CyBioTipTray 96/1000µL (i.e. all wells simultaneously).

Once the automated protocol is complete, the user is instructed to return the lid to the Assay Plate and incubate in a CO_2 incubator.

Results:

Three independent cell viability assays (A, B, C) were performed using the automated method *RealTime-Glo_v1.0.bms* on the CyBio FeliX with the RealTime-Glo™ MT Cell Viability Assay and an 8-step 4-fold serial dilution of Camptothecin (a DNA topoisomerase I inhibitor, inducing apoptosis in a dose-dependent manner). U2-OS cells (human osteosarcoma epithelial adherent cells) were used. The luminescence was recorded at 24, 30 and 48 hours after treatment. Percent viability was calculated and plotted for each assay against the log_{10}*[Camptothecin]* using a five-parameter logistic equation. Regression variables, as well as intra-assay variability and inter-assay variability data, are reported for each independent assay (A, B, C) and each incubation time (Figure 3).



		Assay A	Assay B	Assay C
24h	R squared	0.99	0.99	0.99
	EC50 (nM)	1597 [1462; 1742]	2582 [2257; 3224]	1441 [1293; 1626]
	intra-assay CV%	3.7	4.5	3.6
	inter-assay CV%	6.9		
30h	R squared	1.00	0.97	0.99
	EC50 (nM)	1075 [nd; nd]	1932 [1505; 3527]	1244 [1121; 1393]
	intra-assay CV%	3.9	5.8	3.7
	inter-assay CV%	9.6		
48h	R squared	1.00	0.99	0.99
	EC50 (nM)	550.7 [nd; 607.2]	1047 [920.2; 1189]	617.6 [559.3; 682.8]
	intra-assay CV%	6.4	7.4	6.8
	inter-assay CV%	18.1		

Figure 3. Automated RealTime-Glo™ MT Cell Viability Assays on the CyBio FeliX. The automated method *RealTime-Glo_v1.0.bms* described above was used to plate U2-OS cells (initial density of 5,000 cells/well) with RealTime-Glo™ reagents and an 8-step 4-fold dilution series of Camptothecin (from 40,000nM to 2.44nM). Graphs A, B, C represent for 3 independent assay plates the % Viability (= luminescence/$luminescence_{(Vehicle\ control)}$ x 100 after background subtraction) against the log_{10}*[Camptothecin]*. A five-parameter logistic regression was fit to the data using the GraphPad Prism software. Results represent measurements collected at 24 hours (green), 30 hours (red) and 48 hours (orange) after plating. In the table, R^2 values represent the fit of the regression with actual data. EC50 values (in nM) were estimated using a five-parameter logistic regression and are expressed with their 95% confidence interval (nd: not determined). The intra-assay variability (intra assay CV%) was calculated as follows: $SD_{\%Viability}$ / $mean_{\%Viability}$ x 100, for all Camptothecin concentrations, for each assay individually, where SD is the standard deviation. The inter-assay variability (inter assay CV%) was calculated as follows: $SD(mean_{\%Viability})$ / $mean(mean_{\%Viability})$ x 100, for all Camptothecin concentrations over the three independent assays. Usually accepted intra CV% values are ≤10%, usually accepted inter CV% values are ≤15%.