

# Corning® Gentest™

## MRP/BCRP Transporter Vesicle Uptake IC<sub>50</sub> Assay

### QUICK START GUIDE

Refer to Kit Instruction Manual for detailed procedure.

© 2012, 2013 Corning Incorporated Printed in USA 2/13 CLS-DL-GT-017

CORNING

#### To place an order in the U.S.

Contact BD Biosciences Customer Support at:  
tel: **877.232.8995**, fax: 800.325.9637 or 858.812.8889;  
online: [shop.bdbiosciences.com](http://shop.bdbiosciences.com).

For technical assistance, contact Technical Support at:  
tel: **877.232.8995** or 978.901.7389; fax: 978.901.7491;  
e-mail: [labware@bd.com](mailto:labware@bd.com)

#### Outside the U.S.

Contact your local distributor or visit [bdbiosciences.com/offices](http://bdbiosciences.com/offices) to locate your nearest BD Biosciences office.

**Corning acquired the Discovery Labware Business including the Gentest™ brand.** For additional Corning product, technical, or distributor information, please e-mail us at [CLSTechServ@corning.com](mailto:CLSTechServ@corning.com), visit our website [www.corning.com/lifesciences](http://www.corning.com/lifesciences) or call 800.492.1110. Outside the United States, call 978.442.2200. For information on the acquisition, visit [www.corning.com/discoverylabware](http://www.corning.com/discoverylabware).

CORNING

#### Corning Incorporated

*Life Sciences*  
836 North St.  
Building 300, Suite 3401  
Tewksbury, MA 01876  
t 800.492.1110  
t 978.442.2200  
f 978.442.2476  
[www.corning.com/lifesciences](http://www.corning.com/lifesciences)

#### For Research Use Only. Not for use in diagnostic or therapeutic procedures.

For a listing of trademarks, visit us at [www.corning.com/lifesciences/trademarks](http://www.corning.com/lifesciences/trademarks).  
Corning Incorporated, One Riverfront Plaza,  
Corning, NY 14831-0001

Description	Cat. No.	Substrate	Preincubation		Incubation	
			Temp	Time (Minutes)	Temp	Time (Minutes)
hMRP1	453455	LT <sub>C</sub> <sub>4</sub>	Room Temp	5	Room Temp	3
rMRP1	453510	LT <sub>C</sub> <sub>4</sub>	Room Temp	5	Room Temp	3
hMRP2	453450	LT <sub>C</sub> <sub>4</sub>	37°C	5	37°C	4
		CDDCF	37°C	5	37°C	15
rMRP2	453523	LT <sub>C</sub> <sub>4</sub>	37°C	5	37°C	10
		CDDCF	37°C	5	37°C	15
hMRP3	453520	Estradiol-17β-Glucuronide	37°C	5	37°C	12
		CDDCF	37°C	5	37°C	15
hBCRP	453271	Estrone-3-sulfate	37°C	5	37°C	3
rBcrp	453345	Estrone-3-sulfate	37°C	5	37°C	4
mBcrp	453252	Estrone-3-sulfate	37°C	5	37°C	4

# Corning® Gentest™

## MRP/BCRP Transporter Vesicle Uptake IC<sub>50</sub> Assay

Example for human MRP2 IC<sub>50</sub> Assay using CDCF as substrate for one compound



### Vesicle/Substrate Cocktail

1 mM CDCF in DMSO	13 $\mu$ L
300 mM GSH	15.5 $\mu$ L
Vesicle Uptake Buffer	1693 $\mu$ L
Human/rat MRP2 vesicle	350 $\mu$ L

Assay Sample Preparation

Prepare the serial diluted test compound

Aliquot 59.25  $\mu$ L into columns 2-5, row A-H

Add 0.75  $\mu$ L serial diluted test compound into columns 2-5, rows A-H

Preincubate at 37°C for 5 minutes

Add 15  $\mu$ L of 25 mM ATP into columns 2-3, rows A-H  
Add 15  $\mu$ L of 25 mM AMP into columns 4-5, rows A-H

Incubate at 37°C for desired amount of time

Add 200  $\mu$ L chilled 1X Wash Buffer into all reaction wells

Transfer to rapid filtration system (vacuum manifold)

Wash 6 times with chilled Wash Buffer

DRY THE FILTER PLATE

Add 100  $\mu$ L of 0.1N NaOH into the sample wells and standards curve onto the filter plate, then elute into a new 96 well plate

Read the plate on fluorescence plate reader

Assay Incubation

Stop Assay

Data Acquisition

Serial Dilution of Test Compound

		Comp1 w ATP ( $\mu$ M)		Comp1 w AMP ( $\mu$ M)		Comp2 w ATP ( $\mu$ M)		Comp2 w AMP ( $\mu$ M)		STD Curve (CDCF) (nM)		
	1	2	3	4	5	6	7	8	9	10	11	12
A	-	200	200	200	200	200	200	200	200	200	200	-
B	-	100	100	100	100	100	100	100	100	100	100	-
C	-	50	50	50	50	50	50	50	50	50	50	-
D	-	25	25	25	25	25	25	25	25	0	0	-
E	-	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	-	-	-
F	-	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	-	-	-
G	-	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	-	-	-
H	-	0	0	0	0	0	0	0	0	-	-	-