Description

The Dicer Fluorogenic Assay Kit is a homogeneous 384-well assay designed to measure the double-stranded-RNA processing activity of Dicer for screening and profiling applications. The Dicer Fluorogenic Assay Kit contains enough purified Dicer, substrate, and assay buffer for 384 reactions.

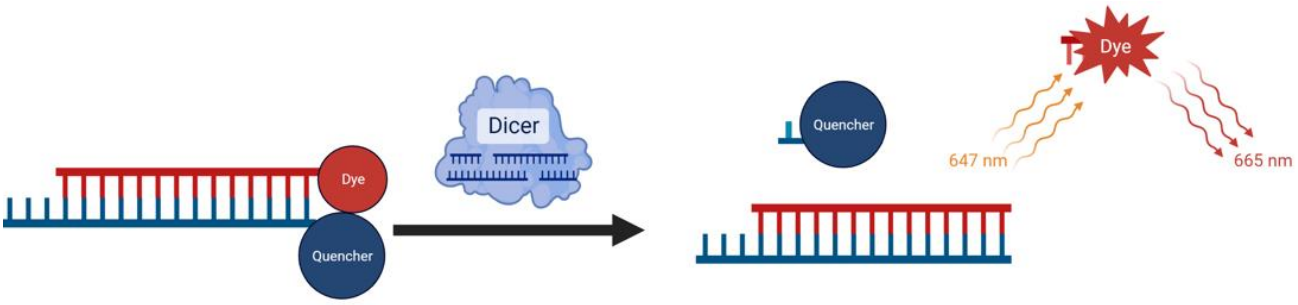

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Figure 1: Illustration of the Dicer Fluorogenic Assay method. The assay relies on an RNA duplex imitating pre-miRNA, containing a conjugated fluorophore and quencher pair. Fluorescence of the dye is effectively quenched by the proximity of the quencher in the intact RNA duplex. The endoribonuclease Dicer processes the pre-miRNA into a mature 22 bp double-stranded miRNA by cleaving the 5' end which is conjugated to the dye, releasing it from the miRNA. Separated from the quencher, the dye emits fluorescence with excitation/emission maxima of λ=647/ 665 nm. Dicer activity is proportional to the increase in fluorescence.

Background

Dicer (also known as endoribonuclease Dicer or helicase with RNase motif) is a ribonuclease that participates in pre-microRNA (pre-miRNA) and double stranded RNA (dsRNA) cleavage. The cleavage of pre-miRNA into mature miRNA and formation of small interfering RNA (siRNA) from dsRNA are essential for gene regulation and DNA repair. Decreased levels of Dicer can result in Age-related Macular Degeneration (AMD), the most common age-related blindness. Dicer impairment is also involved in cancer and RNA-based viral infection. The development of small molecule inhibitors or other strategies that affect Dicer activity can provide new therapeutical avenues for cancer treatment and AMD prevention.

Applications

- Screen molecules that inhibit Dicer RNAse activity in drug discovery High-Throughput Screening (HTS) applications.
- Determine compound IC_{50}.
- Perform Dicer real-time kinetics.

Supplied Materials

Catalog #	Name	Amount	Storage
79083	Dicer, His-Tag (Human)*	45 µg	-80°C
	Pre-micro-RNA Substrate 1	5 µl	-80°C
	DR-01 Buffer	3 ml	-20°C
	384-well black microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

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Materials Required but Not Supplied

- Fluorescent microplate reader capable of measuring excitation/emission maxima of λ=647/ 665 nm
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a "Negative Control", a "Positive Control" and a "Test inhibitor."
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Prepare 1x Assay Buffer by diluting **DR-01 Buffer** 4-fold with distilled water.

2. Thaw **Dicer** on ice. Briefly spin the tube to recover the full content.

3. Dilute Dicer in 1x Assay Buffer to 10 ng/µl (11µl/well - final concentration in the reaction will be 25 nM). Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

   Note: The concentration of Dicer provided may vary. Verify the concentration of the Dicer written on the tube and dilute accordingly. Prepare only the amount required for the assay. Dicer is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted protein.

4. Prepare the Test Inhibitor (4 µl/well): for a titration prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

   4.1 If the Test Inhibitor is water-soluble, prepare 5-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using the 1x Assay Buffer. 1x Assay Buffer is the Diluent Solution.

   Or

   4.2 If the Test inhibitor is soluble in DMSO, prepare it in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute 20-fold in 1x Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO should not exceed 1%.

5. Add 11 µl of diluted Dicer to "Positive control" and "Test inhibitor" wells.

6. Add 11 µl of 1x Assay Buffer to the "Negative Control" wells.

7. Add 4 µl of inhibitor serial dilutions to "Test Inhibitor" wells.

8. Add 4 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

9. Incubate the plate at Room Temperature for 30 minutes.

   Note: If tetracyclines are used as inhibitors of Dicer-mediated processing of pre-microRNAs, Dicer Substrate, not Dicer enzyme, should be preincubated together.

10. Dilute **Pre-micro-RNA Substrate** 1000-fold in 1x Assay buffer to make diluted Dicer Substrate.

11. Initiate the reaction by adding 5 µl of the diluted Dicer Substrate to all wells.

12. Protect your samples from direct exposure to light and incubate at 37°C for 30 minutes.

Component	**Negative Control**	**Positive Control**	**Test Inhibitor**
Diluted Dicer (10 ng/µl)	-	11 µl	11 µl
1x Assay Buffer	11 µl	-	-
Test Inhibitor	-	-	4 µl
Diluent Solution	4 µl	4 µl	-
Incubate at Room Temperature for 30 minutes			
Diluted Pre-micro-RNA Substrate 1	5 µl	5 µl	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

13. Read fluorescence intensity of the samples (lexcitation = 647/10 nm; lemission = 665/10 nm) in a fluorescence plate reader.

Example Results

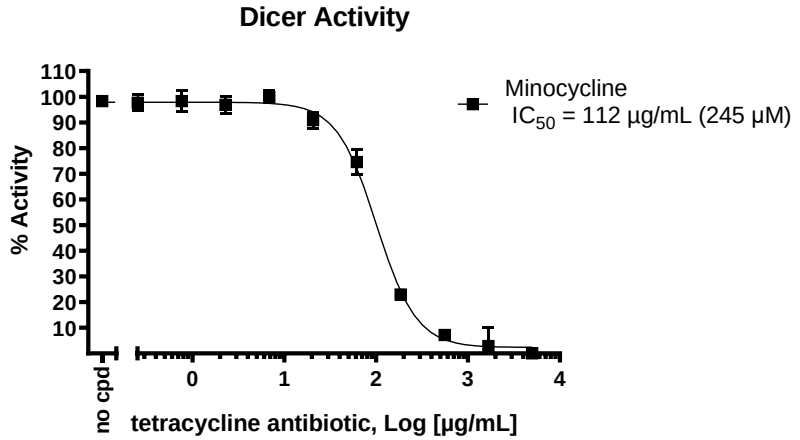

Figure 2. Dicer activity in the presence of a tetracycline antibiotic.
Dicer activity was measured in the presence of increasing concentrations of minocycline hydrochloride (MCE #HY-17412). Results are expressed as percent of control activity (measured in the absence of inhibitor and set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Dicer, His-Tag (Human) Recombinant	79083	20 µg/200 µg
Dicer, Flag-Tag Recombinant	101532	20 µg/100 µg
Human IRE1, His-Avi-tag Recombinant	40709	10 µg