

Double-stranded RNA (dsRNA) Detector

Catalog #RBP-HE101

Product Component	Sizes
dsRNA Detector (1 mg/mL)	200 µg / 1.25 mg

Storage/Transportation Condition Store at -80°C for up to 24 months. Avoid repeated freeze/thaw cycles. Transport on dry ice.

Form Liquid

Storage Buffer PBS, 200mM Arginine, pH8.0

Product Description

Double-stranded RNA (dsRNA) Detector is a GFP-fusion protein that can specifically recognize and bind to dsRNA. This product can be used for dot blot and fluorescence imaging for quantitative analysis of viral dsRNA, non-viral dsRNA, synthesized dsRNA or dsRNA from in vitro transcription. dsRNA detector can be used to measure dsRNA from 0.37 ng to 30 ng.

Recommended Protocol

Prepare the Standards: Make 3-fold serial dilution of poly I:C standards with start concentration at 15 ng/µL, using PBS buffer as a diluent. When pipetting 2 µL of the serially diluted standards, the amount of the poly I:C for each dot in triplicates will be 30, 10, 3.33, 1.11, 0.37 and 0.12 ng. Row 7 is buffer only.

Row#	Amount of poly I:C (ng)	Conc of poly I:C (ng/µL)
1	30	15.00
2	10	5.00
3	3.33	1.67
4	1.11	0.56
5	0.37	0.19
6	0.12	0.06
7	0	0

Dot Blot Protocol

1. Have the nitrocellulose membrane ready. Draw a grid by pencil to indicate the region you are going to blot.
2. Using a narrow-mouth pipette tip, spot 2 µL of standard (poly I:C) and RNA sample onto the nitrocellulose membrane at the center of the grid.

3. Minimize the area that the solution penetrates (usually 2–4 mm diameter) by applying it slowly.
3. Let the membrane dry for 30s – 1 min.
4. Block non-specific sites by soaking in 5% BSA in TBS-T in a 10 cm Petri dish (30 min to 1 h at room temperature).
5. Incubate with dsRNA Detector (final concentration at 1-5 µg/mL) in BSA/TBS-T for 30 min at room temperature.
6. Wash three times with TBS-T (3 x 2 min).
7. Use GFP channel for fluorescence imaging and analysis on iBright1500 (iBrightw CL1500 Imaging System; Cat# A44114).
8. Compare the signal from the unknown sample to that of the standard and estimate the concentration based on fluorescence imaging quantitation.

Assay Data

Dot Blot of poly I:C standards

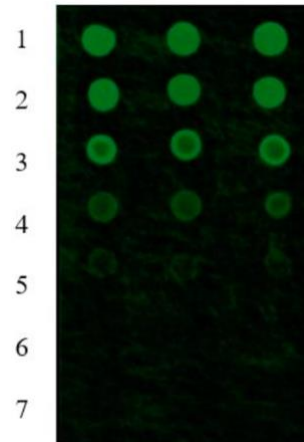


Figure 1. 3-fold serial dilution of poly I:C standards in triplicates (Row 1 to 7). Double-stranded RNA amount larger than 0.37 ug (Row 5) are used for fluorescence quantitation.