EARLY ACCESS PROTOCOL

Lumit® dsRNA Detection Assay

Instructions for Use of
CS355705 and CS355706

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Lumit® dsRNA Detection Assay

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1. Description

The Lumit® dsRNA Detection Assay[a–c] was developed to quantitate dsRNA in biological samples.

2. Product Components and Storage Conditions

PRODUCT	SIZE	PART NUMBER
Lumit® dsRNA Detection Assay	**1 each**	**CS355705**

Includes:

- 15µl dsRNA Standard (CS355701)
- 15µl Lumit® dsRNA Sensor SmBiT (CS355702)
- 15µl Lumit® dsRNA Sensor LgBiT (CS355703)
- 8ml dsRNA Assay Buffer (5X) (CS355704)
- 160µl Lumit® Detection Substrate B (VB405A)

PRODUCT	SIZE	PART NUMBER
Lumit® dsRNA Detection Assay 5X	**1 each**	**CS355706**

Includes:

- 5 × 15µl dsRNA Standard (CS355701)
- 5 × 15µl Lumit® dsRNA Sensor SmBiT (CS355702)
- 5 × 15µl Lumit® dsRNA Sensor LgBiT (CS355703)
- 5 × 8ml dsRNA Assay Buffer (5X) (CS355704)
- 5 × 160µl Lumit® Detection Substrate B (VB405A)

Storage Conditions: Upon arrival, immediately transfer components to a –30°C to –10°C freezer. dsRNA Assay Buffer (5X), once thawed, can be stored at 4°C. The Lumit® dsRNA Sensor SmBiT and Lumit® dsRNA Sensor LgBiT are formulated in 50% glycerol. To prevent repeated freeze-thaw cycles, **do not** store the Lumit® dsRNA Sensors below –30°C to –10°C or in a frost-free freezer. Prior to use, briefly centrifuge tubes to collect contents at the bottom of the tube. Mix by pipetting prior to use.

3. Before You Begin

The components provided are sufficient to test 1 × 96-well plate with one dsRNA standard curve.

Prepare the dsRNA standard curve, Lumit® dsRNA sensors and Lumit® Detection Substrate B on the day of use. A standard curve must accompany test samples for each assay plate. The protocol below describes preparation of reagents sufficient for one 96-well plate. Components contain no preservatives; handle aseptically to avoid microbial and nuclease contamination.

dsRNA Assay Buffer (5X)

Note: Thaw dsRNA Assay Buffer (5X) at room temperature and mix thoroughly prior to use.

Component	Final Concentration	Volume Needed to Make 35ml
dsRNA Assay Buffer (5X)	20%	7ml
nuclease-free water	80%	28ml

Materials to Be Supplied By the User

- nuclease-free water or Barnstead NANOpure® purified water
- white 96-well plate (Thermo Fisher Scientific Cat.# 267350 or Corning® Cat.# 3600)
  Note: Assay is **not** compatible with tissue culture-treated plates.
- lids for 96-well plate (Corning® Cat.# 3098) or adhesive plate sealer
- sterile polypropylene tubes for all sample handling and dilutions
- sterile polypropylene dilution reservoirs with lid (Dilux™ Cat.# D-1002 or USA Scientific Cat.# 3823-3512) for standard curve dilution series
- reagent reservoir trays
- plate shaker for mixing multiwell plates
- plate reader capable of detecting glow-type luminescence from multiwell plates (e.g., GloMax® Discover System, Cat.# GM3000)

4. dsRNA Quantitation Assay Procedure

Note: Prior to use, briefly centrifuge tubes to collect contents at the bottom of the tube. Mix by pipetting.

4.A. Preparing dsRNA Standard Curve

Note: Thaw dsRNA Assay Buffer (5X) and mix thoroughly prior to use. Store at 4°C after initial thaw.

1. Prepare 35ml of dsRNA Assay Buffer by diluting with nuclease-free water.
2. Thaw tube of dsRNA Standard (100µg/ml) and gently mix by pipette.
3. Using sterile polypropylene tubes, prepare serial dilutions to achieve a 10ng/ml solution in dsRNA Assay Buffer as follows (see Figure 1):
   a. Add 10µl of dsRNA to 90µl of dsRNA Assay Buffer (1:10). Mix thoroughly by pipeting.
   b. Add 10µl of dsRNA to 490µl of dsRNA Assay Buffer (1:50). Mix thoroughly by pipeting.
   c. Add 50µl of dsRNA to 950µl of dsRNA Assay Buffer (1:20) to create 10ng/ml dsRNA.

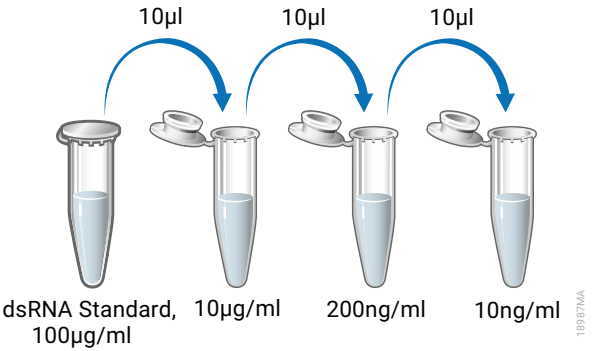

Figure 1. Dilution scheme for dsRNA Standard.

4. To a polypropylene dilution reservoir, add 500µl/well of dsRNA Assay Buffer to wells 1–7.
5. Add 1ml of dsRNA Standard (10ng/ml) to well 8.
6. Prepare samples for an eight-point standard curve by performing serial twofold dilutions (500µl/well) from wells 8–2.
7. Stop after mixing well 2. Well 1 is the negative control (0ng/ml dsRNA).

   Note: To develop a standard curve with your own dsRNA construct, perform a preliminary experiment to determine the actual linear response concentration range. Choose a concentration range that maintains the linear response of the assay. The final linear concentration range may be impacted the presence of modified nucleosides, the absolute determined concentration of sample material, purity and sensitivity of your plate luminometer. See Figure 3 for representation of linear range and luminescence response outside the linear range.

4.B. Preparing Test Samples

Samples containing >10ng/ml of dsRNA should be diluted to ensure they are within the linear range of the assay. If the dsRNA concentration is unknown, we recommend several dilutions (e.g., undiluted, 1:10, 1:100) to ensure one dilution is within the linear range of the assay. When analyzing the data, pick the dilution with the highest luminescence that falls within the linear range of the standard curve. Samples with dsRNA concentrations that are too high will result in reduced luminescence values (see Figure 3).

Note for testing IVT products: In our experience, dsRNA concentrations range from 0.05%–1% within in vitro transcription products with simple purification (e.g., DEAE column). Therefore, we recommend diluting the sample to a concentration of 1µg/ml total RNA, which is likely to result in dsRNA readings within the linear range of the assay (0.16–10ng/ml). **Several dilutions with RNA concentrations higher and lower than 1µg/ml are recommended to be sure the readings fall in the linear range of the assay.**

1. Prepare appropriate serial dilutions of test samples in polypropylene tubes using dsRNA Assay Buffer.

4.C. Adding Samples to Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0									
B	0.16ng/ml	0.16ng/ml	0.16ng/ml									
C	0.31ng/ml	0.31ng/ml	0.31ng/ml									
D	0.63ng/ml	0.63ng/ml	0.63ng/ml									
E	1.25ng/ml	1.25ng/ml	1.25ng/ml									
F	2.5ng/ml	2.5ng/ml	2.5ng/ml									
G	5ng/ml	5ng/ml	5ng/ml									
H	10ng/ml	10ng/ml	10ng/ml									

Figure 2. Recommended plate layout. Use columns 1–3 for the dsRNA standard curve for each plate. Use the remaining wells for test samples.

1. Using an electronic multichannel pipette, add 50µl/well of dsRNA standard curve samples (prepared in Section 4.A) to 96-well white plate.
2. Add 50µl/well of test samples to the same plate.

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4.D. Preparing and Adding dsRNA Sensor Reagents

Prepare the following reagents and use within 5–10 minutes of preparation.

1. Remove the Lumit® dsRNA sensors from freezer and briefly centrifuge. Gently mix each sensor by pipette before use.
2. Dilute Lumit® dsRNA Sensor SmBiT 1:100 with dsRNA Assay Buffer (10µl + 990µl dsRNA Assay Buffer) and mix thoroughly.
3. Dilute Lumit® dsRNA Sensor LgBiT 1:10 with dsRNA Assay Buffer (10µl + 90µl dsRNA Assay Buffer) and mix thoroughly.
4. Complete dsRNA sensor reagent by adding 21.5µl of each sensor to 7ml of dsRNA Assay Buffer. Mix thoroughly by inverting the tube.
5. Add sensor reagent to a reagent reservoir tray. Using a multichannel pipette, add 50µl/well of sensor to sample wells. Place plate with lid on plate shaker and gently shake for 1 minute.
6. Incubate plate for 60 minutes at room temperature.

4.E. Preparing and Adding Lumit® Detection Substrate

Prepare this substrate near the end of the 60-minute incubation (Section 4.D, Step 6). Protect from extended light exposure.

1. Remove Lumit® Detection Substrate B from freezer and mix by pipetting.
2. Add 160µl of Substrate B to 3.04ml dsRNA Assay Buffer and mix by inverting the tube.
3. Add the substrate to a reagent reservoir tray. Using a multichannel pipette, add 25µl/well to samples.
4. Shake the plate for 1 minute on a plate shaker. Protect plate from extended light exposure.
5. After 15 minutes, record luminescence.

5. Data Analysis

1. Determine background by obtaining the average luminescence (RLU) from the 0ng/ml dsRNA standard.
2. Subtract this background RLU from all samples (standard curve and test samples).
3. Use curve fitting software such as GraphPad Prism® to create a dsRNA standard curve using these subtracted values: Plot both y (RLU, subtracted) and x ([dsRNA]) axes in log format.
4. Interpolate test sample concentrations using linear or third-order polynomial regression curve fitting.
5. Multiply the results by the sample dilution factor to determine the concentration of dsRNA in the undiluted sample.

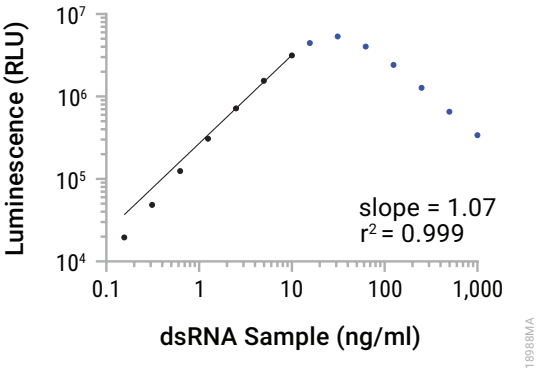

Figure 3. Standard curve response range of the dsRNA Standard, demonstrating linear and nonlinear response concentrations. Luminescence plotted after subtracting backgrounds. This figure is for illustration only. Always perform a standard curve on each experimental plate.