KACTUS

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MDA5 dsRNA Quick-Quantification Kit

Catalog # MD-DS00B (96 tests)

Component #	Component Name	Quantity	Storage
MD-DS00B-01	Lyophilized MDA5 Reaction Mix	12 x 8-well strips	2-8°C
MD-DS00B-02A	dsRNA Standard (100ng/μL) with UTP	100 μL	
MD-DS00B-02B	dsRNA Standard (100ng/μL) with N1-methyl-pseudouridine (m1Ψ)	100 μL	
MD-DS00B-03	Stop Solution	0.5 mL	
MD-DS00B-04	Color Development Buffer A	2 mL	2-8°C, avoiding light
MD-DS00B-05	Color Development Buffer B	10 mL	2-8°C
/	Microplate (clear flat bottom)	1 x 96-well plate	

OTHER EQUIPMENT REQUIRED BUT NOT PROVIDED:

- RNase-free distilled or deionized water
- Mini-centrifuge
- Microtiter plate reader with wavelength capability at 620nm
- Single channel/Multichannel pipettes and sterilized pipette tips.
- Incubator or water batch
- Reagent reservoirs

QUALITY:

Detection range: 0.078ng/μL~5ng/μL
Sensitivity: 0.078ng/μL
Coefficient of Variation (CV): <5%

STORAGE CONDITIONS AND EXPIRATION

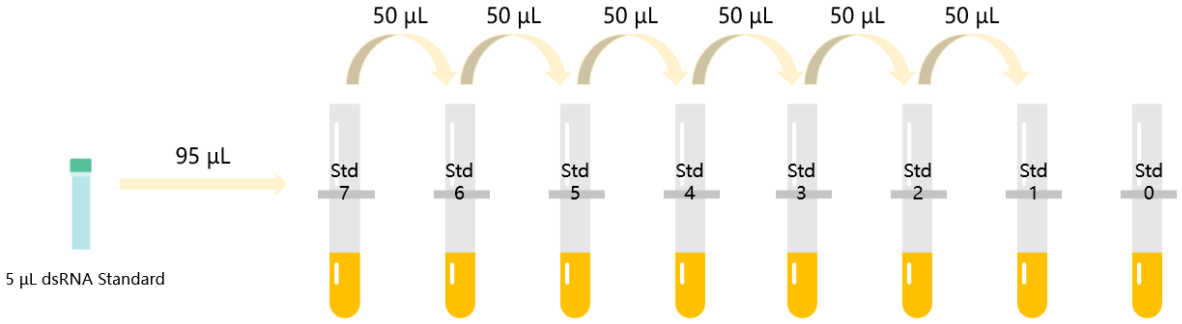
Kits are shipped with blue ice. Store the kit at 2-8°C. The kit remains active for up to 1 year.
Opened dsRNA standard may be stored for up to 2 weeks at 2-8°C. It should be stored at -20°C for long-term usage. Avoid repeated freeze-thaw cycles.

BACKGROUND AND DETECTION PRINCIPLE

MDA5 dsRNA Quick-Quantification Kit is used to detect the presence of undesired dsRNA molecules in IVT reactions or dsRNA in viral/non-viral nucleic acid extracts. MDA5 is an innate immune receptor for double-stranded RNA (dsRNA) with dsRNA-dependent ATPase activity. Single-stranded RNA (ssRNA) doesn't stimulate MDA5 ATPase activity, which enables high specificity for dsRNA detection. Upon dsRNA binding, MDA5 ATPase is activated, converting ATP into ADP+Pi. Then, inorganic phosphate (Pi) reacts with malachite green reagent and forms colored compounds. Colorimetric absorption at 620nm is proportional to dsRNA concentration.

PROTOCOL

1. Equilibrate all reagents and samples to room temperature (18 - 25°C) before use. Keep at room temperature for at least 30 minutes.
2. Prepare standards:
    a. Dilute 5µL of dsRNA standard (100ng/µL) into 95µL of RNase-free ddH2O to get 5ng/µL Standard 7.
    b. 50µL Standard 7 mix with 50µL RNase free ddH2O to get 2.5ng/µL Standard 6. Repeat the dilution of Standard 6 to obtain Standard 5, followed by Standards 4, 3, 2, and 1. The schematic drawing shows the preparation of a series of standards. Blank or Standard 0 is RNase-free ddH2O.



3. Prepare fresh color development buffer:
    a. Working buffer is not stable for storage and should be prepared fresh by mixing buffer A and buffer B at 1:5 (v:v), as shown in the table below.

Reactions	Color Development Buffer A (mL)	Color Development Buffer B (mL)	Working Buffer (mL)
12	0.2	1.0	1.2
30	0.5	2.5	3.0
60	1.0	5.0	6.0
120	2.0	10.0	12.0

4. Reaction:
    a. Add 40µL samples or standards into MDA5 mix in 8-well strip, mix well, and quickly spin down liquid. Incubate at 37°C for 15 minutes.
5. Stop reaction:
    a. Add 5µL stop buffer in each well, mix well, and quickly spin down liquid.
6. Colorimetric assay:
    a. In a new 96-well plate, take 5µL terminated reaction mixture from the previous step and add it into 95µL freshly prepared working buffer. Avoiding light, keep at room temperature for 30 minutes.
7. Read absorbance at 620 nm. Blank using the Standard 0.

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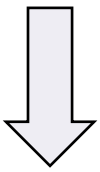
SUMMARY OF PROTOCOL:

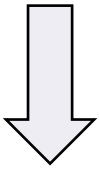
Add 40μL samples or standards into MDA5 mix in 8-tube strip.



Spin down liquid and incubate at 37°C for 15 minutes.

Add 5μL stop buffer in each well. Mix well.

Take 5μL terminated reaction mixture from previous step and add it into 95μL working buffer.



Develop the color for 30 minutes at room temperature, avoiding light.

Read the OD of the microplate at 620nm.

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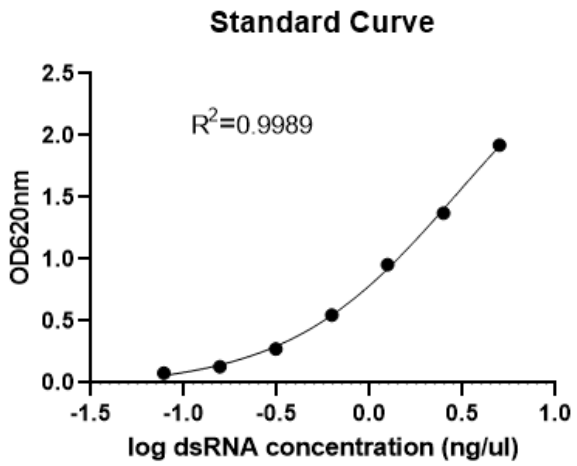
DATA ANALYSIS

1. Standard Curve:
    a. Calculate the mean absorbance for each set of duplicate standards and controls, and subtract the average zero standard optical density. Plot the standard curve using 4-parameter logistic (4PL) curve fitting. Other curve-fitting models are optional.
    b. Example Standard Data:

dsRNA standards	dsRNA Concentration (ng/μL)	OD620 (blank deducted)
Standard 7	5	1.92175
Standard 6	2.5	1.3725
Standard 5	1.25	0.95615
Standard 4	0.625	0.54685
Standard 3	0.3125	0.27495
Standard 2	0.15625	0.13085
Standard 1	0.078125	0.07835
Blank	0	0



2. Determination of dsRNA concentration in samples:
    a. Calculate the mean absorbance for each set of duplicate samples and controls and subtract the average zero standard optical density. Enter the samples' OD620 into the standard curve equation to calculate the dsRNA concentration of the sample.
    b. If the dsRNA concentration of the sample is lower than the detection limit (LOQ) of 0.078ng/μL, then dsRNA is not detected, or the dsRNA concentration is <0.078ng/μL.
    c. The dsRNA concentration of the samples should be within the linear range of the standard curve. If it exceeds the upper limit of the linear range, the sample needs to be diluted and the colorimetric assay redone.

PRECAUTIONS AND RECOMMENDATIONS

1. RNA samples or nucleic acid extracts should contain no phosphate.
2. 1-20μg total RNA from IVT reaction or nucleic acid extracts in 40μL volume is used for one reaction of dsRNA quantification.
3. To ensure data accuracy, don’t use kit components from different batches or different kits.
4. Create standard curve before sample measurement. Each standard is run in duplicate.
5. For samples with unknown dsRNA concentration, multiple dilutions may be necessary to get the OD value in linear range of the standard curve.
6. Avoid light exposure in color development step.
7. Color Development Buffer A contains sulfuric acid. Rinse with plenty of water in case of spillage.
8. MDA5 dsRNA Quick-Quantification Kit is for research use only.