TRIBI SCIENCE

M13 Phage qPCR FAST Titer (100 Tests) Catalog #: TBS4053-100

Product Description

TribioScience's M13 Phage qPCR FAST Titer Kit incorporates TribioScience FAST technology paradigm and Probe Taqman qPCR technology for an accurate, one-step, and time-saving qPCR titration of M13. The probe is labeled with Fluorescence FAM. The entire procedure can be completed within 2-3 hours. With its timesaving 1-minute enzyme activation technology, specificity, and sensitivity, the performance of TribioScience's M13 Phase qPCR FAST Titer Kit is unmatched by similar kits currently on the market.

Kit Content for 100RXN

Component	Volume	Part Number
Fast qPCR mix	1.0 mL	M13001S
M13 Primer-Probe Mix	0.3 mL	M13002
Water	1.0 mL	M13003
M13 Control DNA	20 µL	M13004
DNase I Reaction Mix	0.25 mL	M13005

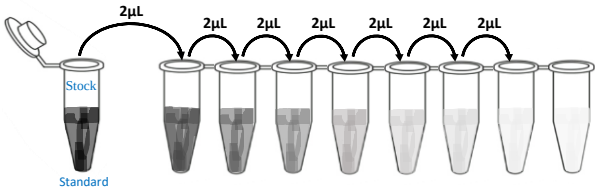
Note: The probe is labelled with Fam.

Storage and Shelf-life

Store at -25ºC to -15ºC immediately upon arrival. Minimize the number of freeze-thaw cycles to ensure superior performance. The Kit is stable for one (1) year from the date of arrival.

Protocol

1. **Phage DNA Sample Preparation:** Phage DNA may have some residual, free DNA which is not from intact phage particles. To remove these DNA (floating or ruptured phage particles), the phage sample is pre-treated with DNase I.
2. **DNase I Treatment recommended for crude phage samples:** Add 2 µL of DNase I into 200 µL of phage sample. Incubate samples at 37°C for15 minutes to digest free gDNA. After then, incubate at 95°C for 10minutes to inactivate DNase I, release phage DNA. The pre-treated phage sample can be used for qPCR amplification.
3. **Standard Control DNA Preparation**: The M13 phage Control DAN is 10^{11} Pfu/mL as stock. Perform 7 serial dilutions of the Standard Control DNA at 10-fold manner by diluting 2 µL Standard DNA into 18 µL Nuclease-Free water in each concentration. Dilutions 10^{11} to 10^{4} Pfu/mL will be used for generating the standard curve. The detail is below:



STD	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Water(µL)		18	18	18	18	18	18	18
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition(µL)		2	2	2	2	2	2	
Std Conc (Pfu/mL)	10^{10}	10^{9}	10^{8}	10^{7}	10^{6}	10^{5}	10^{4}	0

4. **qPCR Preparation:** All reactions are set up on ice in duplications. The reaction volume is 25 µL.

Component	Volume (µL)
Fast qPCR mix	10
M13 Phage Primer-Probe mix	3
DNA Sample or Standard, NTC	3
Water	4
Final Volume	20

Note: water can be used NTC control.

5. **qPCR Running Parameters:** Program the qPCR instrument as follows, and Color Channel is FAM:

Purpose	Temperature (°C)	Time	Cycle(s)
Enzyme Activation	95	60S	1
Denaturation	95	15S	40
Priming/Extension	60	60S	

6. **Titer Calculation**

Plot Ct value (Y-axis, Linear scale) vs. Virus titer (X-axis, Logarithmic scale). Generate a logarithmic regression using the 7 Standard Control DNA dilutions to determine the unknown virus sample titer using y= mx +b for the trendline equation. The R^2 value should be >0.95 to justify the proper assay setup. Note to include the dilution factor in the final calculation.

Use the Ct values to calculate the viral titer of the viral sample with the following formula:

Titer of unknown sample (Pfu/ml) = $e^{(Ctx-b)/m}$, Where m is the slope of the line, and b is the y-intercept.

Example: Trendline equation is y= -1.349ln(x) + 40.898; Ct if unknown sample = 16.98

M13 Phage titer (Pfu/ml) = $e^{(16.98-40.898)/-1.349}$ =5.01 x 10^{7} pfu/ml

Note: Remember to include the dilution factor in the calculation if dilutions of phage particles had been performed.

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