

Lentivirus qPCR Titration Kit

Cat. No.	Quantity	Store at -20°C
VSL-0086	100 reactions	

Assay Description and Application

The Lentivirus qPCR Titer Kit provides a simple, one step lentiviral vector titration. This kit employs a quick RNA extraction step, followed by qRT-PCR assay, which can be completed within 2 hours. This kit is superior in both sensitivity and specificity, and its non-specific background is minimal compared to similar kits.

Kit Components

Virus Lysis Buffer	800µl
Reagent-mix (RT reagent & Primer)	1.0ml
STD1 (lentivirus standard 1)	300µl
STD2 (lentivirus standard 2)	300µl

Additional Materials Required (not provided in the kit)

2x qPCR Mastermix (appropriate for your particular instrument)

Shipping and Storage

Upon arrival, Virus Lysis Buffer, Reagent-mix and STDs should be stored at -20°C. Avoid repeated freeze-thaw cycles to retain maximal performance. Lentivirus qPCR Titer Kit is stable for 1 year from the ship date when stored and handled properly.

Protocol Overview

Viral samples are collected from your virus-producing cell line or purified viral preparations. Employing the virus lysis buffer provided in the kit, viral RNA is isolated from a small aliquot of the sample. RT and primers included in the reagent-mix are then used with the viral RNA, the lentiviral STD1 and STD2 to determine threshold cycle (Ct) values via RT-qPCR. The titer of your samples can be determined by lenti-titer calculation from resulting Ct values.

Protocol

1. Harvest lentiviral supernatant from cells and centrifuge it for 5 min at 2000g to remove cells and debris. (Please omit this step if you purchased the virus.)

NOTE: Please dilute your lentiviral stock 10-100 fold with 1xPBS if the lentiviral sam-

ples underwent concentration and purification procedures.

2. Add 2µl of lentivirus sample to 18µl of virus lysis buffer.
3. Mix 10µl of the sample from Step 2 with 40µl of Reagent-mix. Mix 10µl STD1 with 40µl Reagent-mix, and 10µl STD2 with 40µl Reagent-mix, all in different tubes.
4. Incubate the mixtures at room temperature for 3 min.
5. Prepare the reactions according to the tables below. Please prepare reactions for each unknown sample and STDs in triplicate, and one NTC reaction for negative controls (Reagent-mix without virus) to ensure the system is not contaminated:

	Samples	STDs	NTC
2x qPCR Mastermix	12.5µl	12.5µl	12.5µl
Lentivirus Sample Mixture (from Step 3)	12.5µl	-	-
STD1 or STD2 Mixture (from Step3)	-	12.5µl	-
Reagent-mix	-	-	12.5µl
Total per reaction	25µl	25µl	25µl
Replication	x3	x6	x1

6. Program the real-time qPCR instrument for the following qRT-PCR reaction cycles. (The conditions were optimized on ABI® StepOne™ system.)

Temperature (°C)	Duration	Number of Cycles
42	20min	1
95	10min	1
95	15sec	40
60	1min	

Data Analysis

The titer of your samples can be calculated from Ct values by using the formula:

$$\text{Titer of unknown sample} = 5 \times 10^7 / 2^{3(Ct_{\text{std}} - Ct_{\text{sample}})}$$

Ctx = Average of 3 Ct values of the unknown sample

Ct1 = Average of 3 Ct values of STD1

Ct2 = Average of 3 Ct values of STD2

ATTENTION: Include the dilution factor in the calculation, if samples have been diluted, e.g. 1:100 in Step 1, so the final titer will be $100 \times 5 \times 10^7 / 2^{3(Ct_{\text{std}} - Ct_{\text{sample}})}$.

Recommendations for Optimal Result

- Start the titration assay as soon as the virus is produced or thawed
- Make aliquots of the reagent to avoid contamination
- Start the PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions

*For laboratory research only. Not for clinical applications.
For technical questions, please email us at support1@capitalbiosciences.com*