*baculo*QUANT™ ALL-IN-ONE

This leaflet provides an introductory Guide to using our *baculo*QUANT™ ALL-IN-ONE kit. The kit is supplied under a research use only licence - see end of this Guide for more details.

Product Information

If you require technical assistance, please contact the OET team through info@oetltd.com

Product	Catalogue Number	Size
*baculo*QUANT™ ALL-IN-ONE	100602	100 reactions

Kit Contents and Composition

Product guarantee: 1 year from the date of purchase, when properly stored and handled.

Item	Composition	Storage
Virus Internal Standard	Ac.null virus, 450µl at ~10^8 pfu/mL	Aliquot and store at -20°C
RNAse-free Water	RNAse-free water 2 x 1.9mL vials. For use in qPCR mix, negative control and diluting virus internal standard	Tightly capped at -20°C
DNA Positive Control	*flash*BAC™ DNA 20ng/µL	Tightly capped at -20°C
Primer/probe Mix Lyophilised	Dissolve in 330µL RNAse-free water	Aliquot and store at -20°C
Brilliant II qPCR Low ROX Master Mix (x2 conc.)	1.3mL vial	Aliquot and store at -20°C in the dark
Lysis Buffer	1mL	Aliquot and store at -20°C

Kit Contents and Preparation

Overview

*baculo*QUANT™ ALL-IN-ONE is the revolutionary qPCR based baculovirus DNA extraction and quantification kit from OET's collaboration with Agilent Technologies. Accurate virus titres are essential for determining correct multiplicity of infection and to ensure optimal recombinant protein production. Traditionally baculoviruses have been titrated by plaque assays or antibody based assays that require 3-4 days, however the *baculo*QUANT™ ALL-IN-ONE kit contains reagents to extract viral DNA and determine accurate titres within hours.

Virus titration by qPCR is carried out using probes and primers specific to *gp64*, a gene that encodes the essential virus envelope protein GP64. GP64 is involved in membrane fusion during viral entry and incorporated into budded virus particles during virus egress. Following extraction of virus DNA from freshly amplified* low passage budded virus samples, it is possible to determine the number of virus particles via qPCR amplification using the *gp64* specific probe and primers. The greater the number of virus particles within a sample, the higher the concentration of virus DNA that will be present. By comparing to a known standard curve, it is possible to establish an accurate titre which can be converted to equivalent plaque forming units per mL (Qpfu/mL).

Advantages of using *baculo*QUANT™ ALL-IN-ONE:

- Titre accuracy comparable to plaque assay method
- Less than 1 hour hands-on time with virus DNA extraction and titration in under 3 hours
- Compatible with any AcMNPV-based baculovirus system containing *gp64*
- Single step virus DNA extraction
- qPCR primers/probe provided as a single reagent mix
- Agilent Technologies' high performance, ultra-sensitive master mix reagent included
- Plaque assay titred virus stock included for standard curve generation

**baculo*QUANT™ ALL-IN-ONE extraction and titration system must be used on fresh budded virus stocks (less than 3 months old). After this period, aggregation and degradation of virus particles may occur resulting in misleading Qpfu/mL values. For older virus stocks we recommend using a plaque-assay to titre virus - see our BaculoComplete manual for details and guidance www.oetltd.com/shop

Required by User:

- Recombinant budded virus stock(s) to be analysed - ensure culture medium has been clarified by centrifugation 14,000 rpm for 20 minutes] to remove all traces of cellular DNA and debris - this is normally a standard step when producing budded virus stocks
- PCR thermal cycler with heated lid
- Real-Time PCR Sequence Detection System (SDS)
- Optical 96 well plates and adhesive plate seals
- 96-well plate rotor for low-speed centrifugation
- Ice box
- Sterile 1.5 and 0.2mL microtubes
- Sterile pipette tips

Note: This kit provides reagents sufficient for 100 qPCR reactions. The kit can be used to titrate up to 24 recombinant viruses in triplicate alongside a standard curve in a single run in a 96 well plate or a lesser number of viruses if they are titrated in multiple runs alongside multiple standard curves. Lysis buffer provided is sufficient for 50 virus extractions. The protocol described below has been developed using Applied Systems® 7500 Real Time PCR SDS. We recommend following the manufacturer's instructions when using alternative qPCR systems.

Method:

1. Prepare serial log dilutions (1 in 10) of the virus internal standard using the RNase-free water; both supplied in the kit. These, alongside the undiluted internal standard, will be used to generate the virus standard curve. We suggest using 10µL virus standard (~10^8 pfu/mL) plus 90µL RNase-free water to give ~10^7 pfu/mL. Take 10µL of this solution and add to 90µL RNase-free water to generate ~10^6 pfu/mL. Continue diluting in this way until you have ~10^3 pfu/mL. In total you will prepare 5 tubes with virus ranging from 10^7 to 10^3 pfu/mL plus the undiluted virus standard at 10^8 pfu/mL (so your standard curve will comprise 6 points).

2. Following steps a-e below, extract virus DNA from 80µL of (a) the virus internal standard (~10^8 pfu/mL), (b) each of the five diluted virus standards (10^7-10^3 pfu/mL) and (c) each of the recombinant budded virus stocks that you wish to titrate. You will need a thermal cycler with a heated lid and the supplied lysis buffer.

   a) Set up a thermal cycler to run the following lysis programme and allow to reach temperature;

Step 1:	65°C for 15 minutes	Step 4:	96°C for 1 minutes	Step 7:	20°C hold
Step 2:	96°C for 2 minutes	Step 5:	65°C for 1 minutes		
Step 3:	65°C for 4 minutes	Step 6:	96°C for 30 seconds		

   b) Dispense 80µL of the virus internal standards (1 undiluted and 5 diluted standards) and your unknown virus stocks that you wish to titrate into individual 0.2mL clear microtubes. If you suspect the titre of your unknown sample to be greater than 10^8 pfu/mL, you should dilute it (e.g., 1 in 5 or 1 in 10) so that the titre is within the range of the standard curve.

   c) Centrifuge at high speed (~16,000 x g or 13,000rpm) for 5 minutes in a microcentrifuge. Remove the supernatant fraction carefully and discard into Virkon® or similar disinfectant. Hold the tube in the same orientation as positioned in the centrifuge and remove the supernatant from the opposite side so as not to disturb the virus pellet.

   d) Re-suspend the virus pellet in 20µL of the supplied lysis buffer by briefly vortexing. Place the 0.2mL tubes into the thermal cycler and run the lysis programme.

   e) The DNA is now ready to use for qPCR titration or it can be stored overnight at -20°C.

3. Prepare qPCR reactions on ice as a master mix according to how many viruses you need to titrate. Multiply the amounts shown in the table below by the number of reactions required. We recommend analysing all samples in triplicate. The *baculo*QUANT™ ALL-IN-ONE kit can allow for a maximum of 24 recombinant viruses to be titrated including 2 extra reactions. **Note:** The probe is light sensitive and therefore should be kept in the dark as much as possible.

Reagents	1 qPCR Reaction	Example Master Mix: 1 set of DNA standards (6 x 3) + DNA positive control (1 x 3) + RNAse-free water negative control sample (1 x 3) + 5 unknown virus DNA samples (5 x 3) + 2 extra reactions = 41
qPCR Low ROX Mix (x2) RNAse-free Water (qPCR) Primers + Primers Mix	12.5µL 7.5µL 3µL	512.5µL (12.5µL x 41) 307.5µL (7.5µL x 41) 123µL (3µL x 41)

Total Volume	**23µL**	**943µL**

4. Aliquot 23µL of master mix per reaction into the appropriate number of wells in a 96-well plate. To each set of triplicate wells, add either 2µL of the virus internal standards or unknown viruses, 2µL of the DNA positive control, or 2µL negative control, to give a final reaction volume of 25µL in each well. Seal the 96-well plate according to the manufacturer's instructions.

5. Centrifuge the 96-well plate briefly at low speed to bring the reaction mixtures to the bottom of the wells. Ensure there are no bubbles on the surface of the reactions.

6. Place the 96-well plate within the Real-Time PCR SDS and enter the required information into the software - e.g. the position of each reaction, the fluorescent dyes used (6FAM and IOWA BLACK→FQ) and values of the standard DNA samples (10^8-10^3 pfu/mL). If IOWA BLACK→ FQ is not listed as a standard dye, please select TAMRA. Perform the DNA amplification following the manufacturer's instructions and default cycling conditions as detailed below for the Applied Systems® 7500 Real Time PCR SDS:

	Temperature	Time	Number of Cycles
Enzyme Activation	95°C	10 minutes	1 cycle
Denaturation	95°C	15 seconds	40 cycles
Annealing/Extension	60°C	60 seconds	

7. On completion of the qPCR cycle programme, the most exponential part of each of the amplification curves will have been automatically detected by the SDS software and their C_t values calculated from the default threshold (example shown in Figure 1). Occasionally, this may need to be adjusted manually and the threshold may need to be altered to the most exponential phase of amplification to give improved slope and correlation coefficient values (as described by the manufacturer). However, where possible, baseline setting and threshold levels should remain consistent between assays to improve accuracy and reproducibility.

8. Most SDS software will derive unknown virus titres from an automatically generated standard curve. However, if required export the C_t values into a data analysis program (e.g. Microsoft Excel) and calculate the mean C_t for each virus. Generate a standard curve using the virus internal standard C_t values vs. pfu/mL titre (example shown in Figure 2). Data points that significantly deviate from the trend line should be omitted when plotting the standard curve. Ideally the standard curve should have an R2 value >0.95. If the R2 value is <0.95 then omit the last two dilutions (10^4 and 10^3 pfu/mL) and re-plot the standard curve.

9. Plot the unknown virus C_t values against your standard curve to obtain a pfu/mL value for each virus (Figure 2).

   Note: Standard curve values may differ between Real Time qPCR machines and operators. We recommend carrying out the standard curve each time a virus is titrated using the kit.

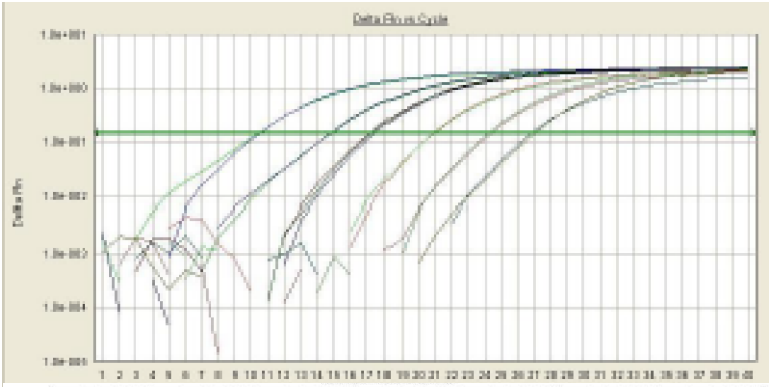

***Figure 1**. Typical amplification plot from ABI's SDS 7500*

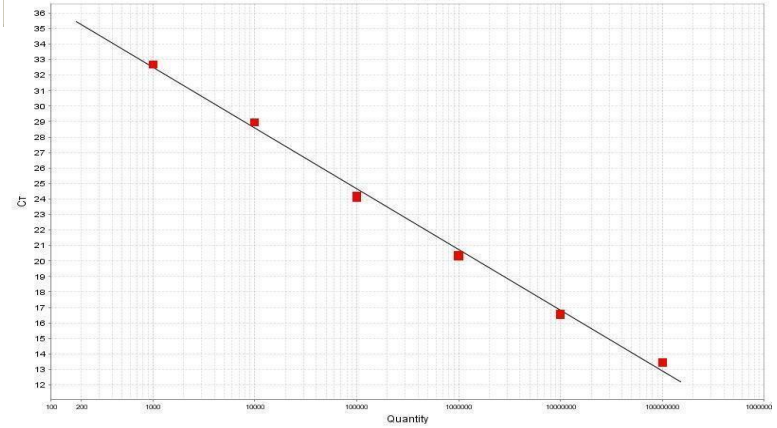

***Figure 2.** Standard curve showing C_t value vs. pfu/mL*

Product Use

Products are for research purposes only. Not for diagnostic or therapeutic use. For applications including the production of proteins for commercial or diagnostic use including clinical/therapeutic use please contact info@oetltd.com.

The use of the licensed probe in this kit is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,538,848, 5,723,591, 5,876,930, 6,030,787, 6,258,569 and 5,804,375. The use of TaqMan™ fluorogenic probes in 5' nuclease assays is covered by U.S. Patent No. 5,210,015 and 5,487,972 owned by Roche Molecular Systems Inc. and by U.S. Patent No. 5,538,848 owned by The Applera Corporation. Purchase of the *baculo*QUANT™ ALL-IN-ONE Titration Kit does not provide a licence to use this patented technology. Further information on purchasing licences to practice this technology may be obtained from Applied Biosystems→, 850 Lincoln Centre Drive, Foster City, CA 94404 or from Roche Molecular Systems Inc., 1145 Atlantic Avenue, Alameda, CA 94501, USA. Diagnostic uses under Roche patents require a separate licence from Roche.

A limited use licence for research use is included with each purchase of a ***baculo*QUANT™ ALL-IN-ONE** kit, which can be downloaded from our website https://oetltd.com/product/baculoquant-all-in-one-virus-extraction-titration-kit/