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EXPRESSION
TECHNOLOGIES

QUICK START: *baculo*QUANT™

QUICK START GUIDE to the *baculo*QUANT™ Virus Titration Kit

A complete and detailed *baculo*QUANT™
Virus Titration manual is available to
download from the OET website at
www.oetltd.com.

**All procedures must be carried out
using aseptic technique.**

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the science of baculovirus expression™

PRODUCTS ARE FOR RESEARCH PURPOSES ONLY, NOT FOR DIAGNOSTIC OR THERAPEUTIC USE

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Provided in the kit:

All reagents and materials provided and referred to in this User Guide are for research purposes only:

- DNA standard [25ng/μl]. Store at -20°C
- Dual labelled probe [2.5μM]. Store at -20°C in the dark
- Forward primers [2.5μM]. Store at -20°C
- Reverse primers [2.5μM]. Store at -20°C

To be provided by user:

- Fresh Budded Virus stock of virus to be titrated by Q-PCR
- Virus DNA extraction kit (e.g. Roche Molecular Biochemicals Viral nucleic acid isolation kit)
- Q-PCR master mix (e.g. Abgene Absolute™ Blue Q-PCR, Low ROX mix)

NOTE: You will also be supplied with a user name and password for use with the online titration facility (available at www.oetltd.com), as given below:

Table 1 – Components for Q-PCR Master mix reaction.

Reagents	1 QPCR reaction	Example (x reactions) 1 set of DNA standards + negative control samples + 5 virus DNA samples + 2 extra reactions*
ABsolute™ Blue QPCR Low ROX Mix	12.5μl	437.5μl
Water	7.5μl	262.5μl
Forward primer (2.5μM)	1μl	35μl
Reverse primer (2.5μM)	1μl	35μl
Probe (2.5μM)	1μl	35μl
Total Volume	23μl	805μl

* It is advised that the user prepares enough Master mix to allow for a few extra QPCR reactions, to ensure sufficient final volume as shown by the example in Table 1.

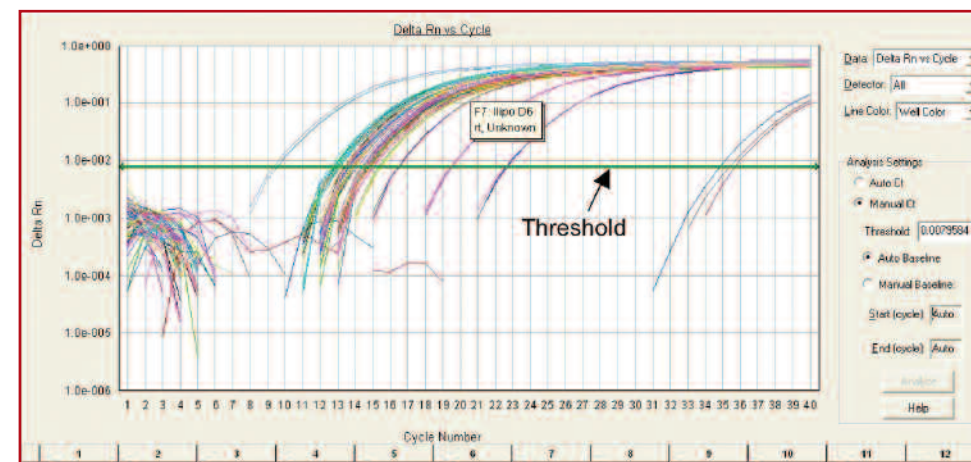
1. Extract DNA from 200μl of budded virus according to the manufacture's protocol using a viral nucleic acid isolation kit. Elute the viral DNA into a final volume of 50μl and store at 4°C until needed.
2. Prepare a log series of 10-fold dilutions of the supplied DNA standard [25ng/μl] in final volumes of 10μl (to give 25, 2.5, 0.25, 0.025, 0.0025ng/μl).
3. Prepare QPCR reactions on ice, as a master mix (See Table 1), according to how many viruses you need to titrate. Multiply the amounts shown in Table 1 (1 QPCR reaction column) by the number of reactions required for the samples and ensure that all reactions are in triplicate. Control reactions contain water in place of DNA and are also carried out in triplicate. For the DNA standards prepare the five

reactions in triplicate as well. For example, if five viruses are to be titred with the given controls and DNA standards, then a minimum of 36 reactions should be set up (with a few extra reactions to allow for errors).

4. Aliquot 23μl of master mix into the appropriate number of wells in a 96-well plate. Remember that all the reactions are in triplicate number.
5. To each set of triplicate wells, add 2μl of the purified viral DNA from step 1, or 2μl each of the five DNA standards (step 2) or control water, to give a final reaction volume of 25μl in each well.
6. Seal the 96-well plate using an adhesive plate seal and plastic spreader.
7. Centrifuge the 96-well plate briefly at low speed to bring the reactions to the bottom of the wells, ensuring that there are no bubbles on the surface.

8. Place the 96-well plate within the Sequence Detection System (SDS 7500) and enter the required information into the software, e.g. the position of each reaction, the fluorescent dyes used (6FAM and TAMRA) and the standard DNA dilutions.
9. Perform the DNA amplification following the manufacturer's instructions.
10. 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 Ct values calculated from the default threshold (see Figure 1). Occasionally, this may need to be adjusted manually and the threshold may need to be moved higher in the exponential phase of amplification to give improved slope and correlation coefficient values.

Figure 1 – Typical amplification plot from ABI's SDS 7500



11. However, where possible, baseline setting and threshold levels should remain consistent between assays to improve accuracy and reproducibility. Ideally, the standard curve should have an R² value >0.95 and give an amplification efficiency of 100% (slope value of -3.322).
 - Export the C_t values into a data analysis program (e.g. Microsoft Excel) and calculate the mean C_t for each virus.

- Go to www.oetltd.com and click the “baculoQUANT” tab and then “Online Service”. Enter both your username and password.
- Enter your first mean ct value and click “calculate”.
- Your virus titre will be displayed in Qpfu/ml and the number of remaining credits will be shown (maximum 26 per kit)