EU VII FP PROJECT "VITAL"

STANDARD OPERATING PROCEDURE

SOP VITAL 023

Protocol for the establishment of IAC incorporation, optimised for each laboratory

CREATED:	REVISED:	APPROVED:
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The internal amplification controls (IACs) which are supplied by Yorkshire Bioscience Ltd. are thoroughly tested before dispatch, including quantification. Quantification by its very nature however is very rarely 100% accurate. Added to this is the fact that we have to dilute our IACs many times to reach the desired concentration. A consequent potential problem is that the desired concentration may not be replicated precisely each time a dilution series is performed on each separate batch of IAC.

In order to resolve this potential problem, all laboratories should, on receipt of each new batch of IAC (e.g. Adenovirus IAC or Norovirus IAC) perform a very simple test to determine the lowest consistent limit of IAC detection:

1. Dilute the supplied IAC solution to 10^{-10} using nuclease free water with 0.1 mg/mL of BSA added.

2. Prepare a mastermix for 11x reactions. Do not add IAC to this mastermix. Replace the volume of target as quoted in the relevant protocol, with the equivalent volume of water.

3. Add *n** ul of each IAC dilution from step 1, to 10 reactions. To the 11^{th} reaction, instead of the IAC, add the same amount of water (negative control).

   *The volume of IAC which is specified in the relevant protocol.

4. Perform PCR. If it is a nested PCR, perform both rounds.

5. Choose the highest dilution of IAC which produces a positive signal.

6. Prepare a mastermix for 4 reactions. Again, do not add IAC to this mastermix. Replace the volume of target as quoted in the relevant protocol, with the equivalent volume of water.

7. Add *n* ul of the dilution chosen in step 5 to the first (1^{st}) reaction.

8. Add *n* ul of the next highest dilution (e.g. if the dilution chosen in step 5 is 10^{-6}, then use 10^{-7} in this step) to the 2^{nd} reaction.

9. Add *n* ul of the next lowest dilution (e.g. if the dilution chosen in step 5 is 10^{-6}, then use 10^{-5} in this step) to the 3^{rd} reaction.

10. In the 4^{th} reaction use water instead IAC dilution sample

11. Perform PCR. If it is a nested PCR, perform both rounds.

12. Repeat steps 6-11 twice more.

13. Choose the highest dilution of IAC which consistently gives three positive signals from the three separate mastermixes. This is the working dilution of IAC which is to be used in all your subsequent tests which include this particular batch of IAC.

14. Please make sure to record against each batch of IAC which final dilution was used for quality control purposes, and so the data can be used at a later date when it comes to publishing.

15. Repeat this procedure for each new batch of IAC delivered to your laboratory, and at convenient intervals to ensure the IAC is at the optimal working concentration.

16. Store the IACs at -70^{o}C.