

Bid Submission Form
Participation in Assignment of Particle Concentration
RFP 8.0

Please complete the following fields:

Contact Information – RFP 8.0

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***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- Determination of Infectious Titer
- Short-term/Field Stability Studies
- Long-term Stability Study
- Other Characterization
- Donation of Supplies/Other Services for Characterization Phase

Each laboratory submitting a proposal should provide a statement describing their experience and capacity to perform the spectrophotometric particle assay described in the standard operating procedure. The statement should specifically address:

- the qualifications of the personnel involved in performing the procedure and reviewing the data,
 - All staff are qualified to graduate, or above, academic level and all have a minimum of 2yr experience in support of a current Cobra Therapeutics clinical programme using an Adenovirus vector generated in PER.C6.
- the equipment that will be used and its calibration status,
 - Spectrophotometer (Beckman DU650i, fixed slitwidth)

This instrument is serviced on an annual basis. A trained and qualified engineer ensures optimal operation of the optical bench. On a weekly basis the internal performance verification routine ensures that the wavelength drive is accurate and repeatable, the available resolution is sufficient, the baseline noise is low and that there is no significant wander of the baseline. All parameters are tested against a specification set by the manufacturer. In addition to the programmed tests spectra are taken for Holmium and Didymium oxide filters to monitor any gross changes in peak position.

Class II biological safety cabinets (Envair)

These are serviced every 6 months by a qualified service engineer.

Variable volume pipettes (Gilson)

All pipettes are cleaned and calibrated every 6 months to a specification set by the manufacturer.

- how long it will take the laboratory to perform the procedure, and review and report results back once the sample is received,
 - 1 – 2 weeks from receipt to report
- the laboratory's readiness to begin testing in mid to late September 2001.
 - Lab ready and available for September 2001

For laboratories wishing to also submit a proposal to perform additional methods of determining the particle concentration, the proposal should include:

- the amount of Ad5 WT Reference Material that will be required to perform the proposed analysis,
 - The particle concentration by Picogreen DNA method will require one sample, equivalent to that supplied for the Spectrophotometric Analysis.

- a complete description of the method, preferably in the form of an operating procedure
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PRINCIPLE :

The PicoGreen™ reagent is a sensitive fluorescent nucleic acid stain, which shows 1000 fold fluorescence enhancement on binding to double stranded DNA. The dye is specific for double stranded DNA thereby minimising any interferences from single stranded DNA or RNA.

Sample fluorescence is compared against that of a standard curve prepared from linear DNA of known concentration (OD_{260nm}). A line of best fit is applied to the standard curve and any unknowns are calculated from knowledge of the slope and Y intercept of the standard curve.

EQUIPMENT:

A Perkin Elmer LS50B Fluorimeter (or similar) capable of excitation at ~ 480nm and detection at ~ 520nm.

1 cm pathlength, (4ml) cuvettes.

REAGENTS:

- 1 20x TE Solution (10mM Tris-HCl, 1mM EDTA, pH 7.5)
- 2 Lambda DNA Standard or similar.
- 3 PicoGreen Dye concentrate.
- 4 Proteinase K at 4 units/ml.

Items 1 to 3 are supplied in the PicoGreen™ kit

PROCEDURE:

Assay Buffer Preparation:

Prepare a working solution of 1x TE from the 20x stock TE. A 4 unit/ml solution of Proteinase K in 1 x TE is required.

Reagent Preparation:

Just prior to use, prepare an aqueous working solution of the PicoGreen Reagent by making a 1:200 dilution of the concentrated DMSO solution in 1x TE/Proteinase K. Prepare enough solution to add 1.5mls to each sample/standard.

DNA Standard Curve:

Prepare a 200ng/ml working solution of dsDNA from the 100µg/ml stock. From the working solution prepare the following concentrations in duplicate:-

Concentration (ng/ml)	Dilution	Vol of Std Added (µl)	Vol of 1x TE/Pro K Added(µl)
50	1:4	375	1125
40	1:5	300	1200
30	1:6.7	224	1276
20	1:10	150	1350
10	1:20	75	1425
5	1:40	37.5	1462.5
1	1:200	7.5	1492.5
0	-	0	1500

Sample Calculations:

$$\mu\text{g/ml Linear DNA} = \text{p/ml}(\text{OD}_{260\text{nm}})/1.1\text{E}12 * 0.02$$

Dilute to 2 different concentrations with 1x TE/Pro K:-

~40 ng/ml and ~20 ng/ml

Because the UV Assay can be an over estimation of the Particle number, normally the results for these dilutions fit on the Standard curve.

Sample Preparation:

At least 1.8mls of sample is prepared (in duplicate) at each dilution (prepared by weight) and the samples heated at 56 °C for 90 minutes.

Assay Setup:

1.5mls of standard/sample was added to a 4ml plastic cuvette. 1.5mls of PicoGreen Reagent is added to each cuvette, the solutions mixed and left at room temperature to incubate for at least 30 minutes prior to fluorescence measurement.

Calculation procedure.

Calculate the actual standard DNA concentrations and plot against the mean fluorescence value obtained from each point. Determine the slope, intercept and correlation coefficient for the line obtained. For each sample the calculation of particles/ml is as follows;

$$p/ml = (A-B)/C *DF/1000*0.02*1.1E12$$

- A = Sample fluorescence
- B = Intercept of calibration line
- C = Slope of the calibration line
- DF = Dilution factor
- 0.02 = Converts $\mu\text{g/ml}$ to OD units
- 1.1E12 = p/ml equivalent of 1 OD unit in a 1 cm pathlength cuvette

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- the laboratory's experience in performing the proposed procedure,
 - The procedure has been used to support entry into a UK clinical trial and to provide data as part of the ongoing stability study (currently at 2yr) that is reviewed by UK MCA.
- the qualifications of the personnel involved in performing the procedure and reviewing the data,
 - All staff are qualified to graduate, or above, academic level and all have a minimum of 2yr experience in support of a current Cobra Therapeutics clinical programme using an Adenovirus vector generated in PER.C6.
- the equipment that will be used and its calibration status,
 - Perkin-Elmer LS-50B Fluorimeter (variable slitwidth)

This instrument is serviced on an annual basis. A trained and qualified engineer ensures optimal operation of the optical bench. Performance verification takes place on a weekly basis, firstly the signal to noise ratio is tested using an internal "Raman test" utilising a sealed water standard. Secondly excitation and emission wavelength accuracy/repeatability is tested using a set of fluorescence standards.

Mettler AT261/AG204 Analytical Balance

This instrument is serviced on an annual basis by a trained and qualified engineer.

Performance verification takes place on a weekly basis using calibrated weights. Just prior to each weighing operation an internal verification procedure confirms correct operation of the balance.

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