EIGC.005_Quantitation with Fluorescence

Standard Operating Procedure Staff Review Page:
I have read and understand the procedure listed above.

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<th>Employee name</th>
<th>Date SOP review complete</th>
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Standard Operating Procedure Approval Page:

Date Implemented: 01 April 2006

Updated: 
Name: Ashima Amin
Date: 08 April 2016

Supersedes: 
Name: Malania Wilson
Date: 12 March 2013

Annual Review and Approval

Michael Zwijck, PhD
Laboratory Director

Changes Made:
040816 – Annual Review, Employee no longer a part of Emory Integrated Genomics Core.
Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Test Principle: Fluorescence quantitation will be performed following DNA or RNA isolation. DNA and RNA concentrations will be used for quality control purposes and be provided to investigators for future use. DNA concentration is determined using PicoGreen staining of the DNA. RNA concentration is determined using RiboGreen staining of the RNA. Briefly, a small sample of isolated DNA or RNA will be diluted in TE. A PicoGreen or RiboGreen solution will be added to the diluted sample plates and read using plate reader.

Specimen Type: Isolated DNA or RNA

Safety:

All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook).

No data are available addressing the mutagenicity or toxicity of PicoGreen. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen. The PicoGreen and RiboGreen solutions are in dimethylsulfoxide (DMSO) so should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Quality Control:

Standards are included with each run. Five out of seven standards must be within range for the data to be passed. The $r^2$ value of the standard curve should not fall below 0.97. If $r^2$ value is lower than 0.97 then samples and standards are replated and quanted again. If $r^2$ value fails a second time new standards are made. When $r^2$ values fail 2 times then issues are logged in the Quality Management file.

Protocol:

Carefully choose which quantitation is appropriate for the samples.

- Quant-iT PicoGreen. 250 pg/mL to 1,000 ng/mL dsDNA
- Quant-iT Broad Range 2-1000 ng
- Quant-iT High Sensitivity 0.2-100 ng
- Quant-iT RiboGreen High-Range 20 ng/mL to 1 ug/mL
- Quant-iT RiboGreen Low-Range 1 ng/mL to 50 ng/mL
Quant-iT PicoGreen Quantitation of DNA

Required Reagents:

20X TE Invitrogen – Cat# T11493
Quant-iT PicoGreen Kit Invitrogen – Cat# P7589
Black 96-well plates Greiner BioOne – Cat# 655076

Protocol summary:

1. Equilibrate assay components to room temperature.
2. Prepare a 2 ug/mL stock solution of DNA standard in 1xTE. Dilute λDNA (Component C) 50-fold.
4. Make the working solution by diluting Quant-iT™ PicoGreen reagent into 1xTE 200-fold (1ul in 198ul).
5. Load 98 ul of 1xTE into each sample well.
6. Add 2 μL of each unknown DNA sample to separate wells and mix well.
7. Add 100 μL of each of the λDNA standards to separate wells and mix well.
8. Load 100μL of working solution in each microplate well.
9. Incubate 2-5 minutes.
10. Measure fluorescence using microplate reader (excitation/emission maxima ~480/520 nm).
11. Use a standard curve to determine DNA amounts.

Setting the standard curve:

<table>
<thead>
<tr>
<th>Dilution (μg/ml)</th>
<th>Method</th>
<th>[ ] with PicoGreen</th>
<th>Acceptable Range (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>20 μl stock + 980 μl 1X TE</td>
<td>1 μg/ml</td>
<td>0.998-1.003</td>
</tr>
<tr>
<td>1.5</td>
<td>15 μl stock + 985 μl 1X TE</td>
<td>0.750 μg/ml</td>
<td>0.745-0.755</td>
</tr>
<tr>
<td>1.0</td>
<td>10 μl stock + 990 μl 1X TE</td>
<td>0.500 μg/ml</td>
<td>0.495-0.505</td>
</tr>
<tr>
<td>0.5</td>
<td>5 μl stock + 995 μl 1X TE</td>
<td>0.250 μg/ml</td>
<td>0.200-0.300</td>
</tr>
<tr>
<td>0.2</td>
<td>100 μl 2 μg / ml + 900 μl 1X TE</td>
<td>0.100 μg/ml</td>
<td>0.098-0.102</td>
</tr>
</tbody>
</table>

See EIGC.005_Appendix_A for detailed protocol process. Use 200ul volumes for 96-well plates. There are no deviations from published protocol. (MP 07581 Revised: 10-June-2008).
Quantitation of dsDNA using Quant-iT Broad Range

Required Reagents:

Quant-iT Broad Range Kit
Invitrogen – Cat# Q-33130

Black 96-well plates
Greiner BioOne – Cat# 655076

Protocol summary:

1. Equilibrate assay components to room temperature.
2. Make the working solution by diluting Quant-iT™ dsDNA BR reagent 1:200 in Quant-iT™ dsDNA BR buffer.
3. Load 200µL of working solution in each microplate well.
4. Add 10 µL of each of the Quant-iT™ dsDNA BR standards to separate wells and mix well.
5. Add 1–20 µL of each unknown DNA sample to separate wells and mix well.
7. Use a standard curve to determine DNA amounts

Setting the standard curve:

In order to obtain accurate DNA concentration reading, a standard curve must be run using DNA solutions of known concentrations. Each BroadRange kit comes with λDNA standards at a range of concentrations (0, 5, 10, 20, 40, 60, 80, and 100 ng/ul). A new standard curve must be run with each plate. Five out of six standards must be within range to pass.

See EIGC.005_Appendix_B for detailed protocol process. Use 200ul volumes for 96-well plates. There are no deviations from published protocol. (MP 33130 Revised: 28-February-2007).
Quantitation of dsDNA using Quant-iT High Sensitivity

Required Reagents:

Quant-iT High Sensitivity Kit  
Invitrogen – Cat# Q-33120

Black 96-well plates  
Greiner BioOne – Cat# 655076

Protocol summary:

1. Equilibrate assay components to room temperature.
2. Make the working solution by diluting Quant-iT™ dsDNA HS reagent 1:200 in Quant-iT™ dsDNA HS buffer.
3. Load 200μL of working solution in each microplate well.
4. Add 10 μL of each of the Quant-iT™ dsDNA HS standards to separate wells and mix well.
5. Add 1–20 μL of each unknown DNA sample to separate wells and mix well.
7. Use a standard curve to determine DNA amounts

Setting the standard curve:

In order to obtain accurate DNA concentration reading, a standard curve must be run using DNA solutions of known concentrations. Each High Sensitivity kit comes with 6DNA standards at a range of concentrations (0, 0.5, 1, 2, 4, 6, 8, and 10 ng/μl). A new standard curve must be run with each plate. Five out of six standards must be within range to pass.

See EIGC.005_Appendix_C for detailed protocol process. Use 200ul volumes for 96-well plates.
There are no deviations from published protocol. (MP 331020 Revised: 28-February-2007).
Quantitation of RNA using Quant-iT RiboGreen

Required Reagents:

20X TE Invitrogen – Cat# T11493
RiboGreen Kit Invitrogen – Cat# R11490
Black 96-well plates Greiner BioOne – Cat# 655076

Determine whether you wish to perform the high-range assay (20 ng/mL to 1 μg/mL RNA) or low-range assay (1 ng/mL to 50 ng/mL RNA), or both.

Protocol summary:

1. Equilibrate assay components to room temperature.
2. Prepare a 2μg/mL solution of RNA standard in 1xTE. Dilute ribosomal RNA (Component C) 50-fold. Further dilute 20-fold to make 100 ng/mL for low-range.
3. Prepare serial dilutions for standard curve. See tables. Prepare fresh each time
4. Make the working solution by diluting Quant-iT™ RiboGreen reagent into 1xTE 200-fold for high-range and 2,000-fold for low-range assay.
5. Load 98 μL of 1xTE into each sample well.
6. Add 2 μL of each unknown RNA sample to separate wells and mix well
7. Add 100 μL of each of the ribosomal RNA standards to separate wells and mix well.
8. Load 100μL of working solution in each microplate well.
9. Incubate 2-5 minutes.
10. Measure fluorescence using microplate reader (excitation/emission maxima ~480/520 nm).
11. Use a standard curve to determine RNA amounts

Setting the standard curve:

For a standard curve, we commonly use 16S and 23S ribosomal RNA, although any purified RNA preparation may be used. It is sometimes preferable to prepare the standard curve with RNA similar to the type being assayed. Most single-stranded RNA molecules yield approximately equivalent signals. Choose either the high-range (20 ng/mL to 1 μg/mL) or low-range (1 ng/mL to 50 ng/mL) standard curve that is appropriate for the sample.
### High-Range Standard Curve

<table>
<thead>
<tr>
<th>Volume (ul) of 1xTE</th>
<th>Volume (ul) of 2 ug/ml RNA Stock</th>
<th>Volume (ul) of 2000-fold Diluted RiboGreen Reagent</th>
<th>Final RNA Concentration in Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>1 ug/mL</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>100</td>
<td>500 ng/mL</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>100</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td>98</td>
<td>2</td>
<td>100</td>
<td>20 ng/mL</td>
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<tr>
<td>100</td>
<td>0</td>
<td>100</td>
<td>blank</td>
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</table>

### Low-Range Standard Curve

<table>
<thead>
<tr>
<th>Volume (ul) of TE</th>
<th>Volume (ul) of 100 ug/ml RNA Stock</th>
<th>Volume (ul) of 2000-fold Diluted RiboGreen Reagent</th>
<th>Final RNA Concentration in Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>50 ng/mL</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>100</td>
<td>25 ng/mL</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
<td>100</td>
<td>5 ng/mL</td>
</tr>
<tr>
<td>98</td>
<td>2</td>
<td>100</td>
<td>1 ng/mL</td>
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<tr>
<td>100</td>
<td>0</td>
<td>100</td>
<td>blank</td>
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</table>

See EIGC.005_Appendix_D for protocol process. Use 200ul volumes for 96-well plates. There are no deviations from published protocol. (MP 11490 Revised: 10-June-2006)
Reading the Nucleic Acid concentration

Open "Magellan v 7.1" Software on the Tecan Infinite M200 Pro.

Create / Edit a Method

Open Template for Assay you are running. C:\users\public\Documents\Tecan\Magellan\mth – Templates DO NOT EDIT-DO NOT SAVE)

Choose Measurement Parameters

   Plate Layout – Fill wells with samples (SM-sample, BL-blanks, ST-standards).
   Make sure dilutions are correct and standards have the correct concentrations
   Standards Curve should be set to linear regression.

Save template with Project ID, order number, and date.

Finish and Run.

Place the incubated black plate in the plate reader drawer then Read.

When all samples have been read, scan in aliquot IDs into the excel file. Please make sure you enter these quants on the QC report. These concentrations can also be added to the LIMS ID’s by ‘modifying their aliquots’ and entering the values into the Concentration field.

Benefits and Limitations:

This procedure requires at least 3 μl of extracted DNA/RNA solution.

It is recommended that all fluorescent solutions be used within a few hours of its preparation, and that it be covered with foil as the fluorescent reagent is susceptible to photodegradation.

Troubleshooting:

See user’s manual