STANDARD OPERATING PROCEDURE

Use this form to document the Health and Safety information associated with the procedure.

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| **Procedure Title** | | DNA quantification | | | | | | |
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| **Dept** | ABE | |  | **Bldg/Rm** | 4210 Sukup |  | **Supervisor** | Adina Howe |

**Procedure Overview** (brief description of the project)

To describe the process of quantifying DNA fluorometrically using Quant-iT™ dsDNA High-Sensitivity Assay Kit and the plate reader or the Qubit. The Qubit should be used for quantification if you have a small number of samples and the plate reader if you have a large number.

**Health and safety information for materials used (**briefly describe the hazards associated with the materials and/or equipment **OR** document your hazard assessment in Section I)

The Quant-iT dsDNA HS reagent is flammable; keep away from heat, hot surfaces, sparks, open flames and other ignition sources.

**Hazard Control Measures**

Please select which type of lab coat, eye protection, and hand protection will be used (Lab coat, eye and hand protection, and closed toe/heel shoes must be selected as required by Section D of the ISU Laboratory Safety Manual.)

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|  | Latex gloves |  | Insulated gloves |  | Face shield |  | Respirator |
|  | Nitrile gloves |  | Safety glasses |  | Lab coat |  | Fume hood |
|  | Neoprene gloves |  | Vented goggles |  | Apron |  | Biosafety cabinet |
|  | Vinyl gloves |  | Splash goggles |  | Dust mask |  | Glove box |
|  | Fully enclosed shoes | | |  | Flame resistant lab coat | | |

***Other Control Measures***

**Methods** (Include step by step instructions detailing the process or attach this document to an existing method.)

1. Bring the buffer, reagent, and standards to room temperature. The buffer can take several hours to warm up depending on its volume. This reaction is very temperature sensitive, so it is important to always have everything at room temperature before any fluorescence readings.
2. Calculate the number of reactions and the amount of working solution you will need.
   1. Calculate the number of reactions you need for quantification by taking the number of your samples, adding the number of standards needed, and adding some overage (typically 10-20%).
   2. The number of standards you need depends on your method and number of samples.
      1. If you are using the Qubit, only one tube each of the lowest and highest standards (0 ng/µL and 10 ng/µL) are needed.
      2. If you are using the plate reader, you will need all 8 standards on each plate and for the last column of the last plate (e.g. if you have 144 samples, it will be 2 plates and you will need 3 x 8 = 24 wells for standards).
   3. You will need 200 µL of the working solution for each reaction.
3. Clean workbench surface with 70% ethanol.
4. It is not required, but it is recommended, to dilute your samples 1:10 with PCR quality water. Make sure your samples are thawed and ready to go before the next step as the fluorescence potential of your working solution decreases over time.
5. Prepare your working solution by diluting the **Quant-iT dsDNA HS reagent 1:200 in Quant-iT dsDNA HS buffer**.
   1. This should be done in an autoclaved 2 mL tube for smaller volumes or a new 15- or 50-mL centrifuge tube for larger volumes. For very large volumes, multiple 50 mL tubes may be needed. Do not use glass containers.
   2. Add the buffer to the tube (200 µL per reaction). Set a pipette to the amount of reagent needed (1 µL per reaction). Remove that amount of buffer from the tube, then add the reagent. Cap and mix thoroughly.
6. Pour the working solution into a sample reservoir if using a multi-channel pipette.
7. Add 190 µL of the working solution into each standard tube or well and 195 µL of the working solution into each sample tube or well.
   1. Qubit uses Qubit assay tubes.
   2. The plate reader uses black, optical 96 well plates.
8. Add your DNA to each tube or well.
   1. For the **standards, add 10 µL** to each tube or well.
      1. When adding standards to plates, they are added to the first column of each plate and after the last column of samples.
      2. This can get confusing if you have full 96 well plates of samples. It is recommended that the first column of each plate is replaced with standards and is instead moved to the end of the last plate. That way column 2 of DNA will still go in column 2 of your optical plate.
      3. Add the standards to the plate in sequential order with lowest DNA concentration in row A to most concentrated in row H.
      4. Qubit requires only two standards: 0 ng/µL and 10 ng/µL.
   2. For your **samples, add 5 µL** to each tube or well.
      1. 1-20 µL is in the original protocol. It was determined that 5 µL was a good baseline for our lab. If some samples give a very low reading, you can repeat them with a higher volume of DNA.
   3. Don’t forget to account for the difference in DNA volumes when doing your calculations afterwards! (Multiply your concentration by 2 to compensate for the difference between the 10 µL standard volume and 5 µL sample volume.)
9. Mix well.
   1. You can mix wells on the plate by either pipetting or by sealing the plate and carefully vortexing. Be sure to spin down the plate afterwards if you choose the second option.
   2. You can mix tubes by either flicking them a few times with your fingers or by gently vortexing. Be sure to spin them down afterwards.
10. Incubate on the benchtop for 2 minutes.
11. Take your prepared samples to the appropriate instrument to quantify your DNA.
    1. For plates
       1. Turn on the plate reader and open the program Gen5 on the plate reader computer.
       2. In the Task Manager window, open DNA\_quantv2.
       3. Load your plate.
       4. Click the Read New button and follow directions.
    2. For Qubit
       1. Place the Qubit in an appropriate work area and plug it in.
       2. Follow the prompts on the screen that describe your situation.
       3. Add your information to the Qubit’s logbook.
12. Tidy workbench, dispose of all waste, and wipe work surface with 70% ethanol.
13. Calculate your DNA concentrations based on the standard curves generated with the DNA standards. It is recommended that the R2 of the standard curve be at least 0.90.

**Waste Disposal Procedures**

Leftover working solution can be capped in its tube and thrown away. All pipette tips should go in the plastic sharps waste container. Used tubes and optical trays can also be disposed of in the plastic sharps waste container for convenience (as they are autoclaved).

**First Aid Procedures**

**Skin contact:** Rinse skin with water. Immediate medical attention is not required.

**Eye contact:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

**Ingestion:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

**Inhalation**: Not expected to be an inhalation hazard under anticipated conditions of normal

use of this material. Consult a physician if necessary.

**Notes to Physician**: Treat symptomatically.

**Most important symptoms and effects, both acute and delayed:**

H227 - Combustible liquid

**Indication of any immediate medical attention and special treatment needed:**

None.

**Spill/Release Containment, Decontamination, and Clean Up Procedures**

Small spills may be absorbed by paper towels and waste from the cleaning procedure may be disposed of in the normal trash. Wipe contaminated surfaces with 70% ethanol. This procedure is unlikely to generate a large spill due to the small volumes used.

**Using Substances Requiring Special Procedures?** No  Yes

(If Yes; identify authorized personnel, designate a use area and specify specialized safety precautions here. Refer to Section B in the ISU Laboratory Safety Manual for details.)

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| **Written By** |  |  | **Date** |  |
|  |  |  |  |  |
| **Approved By** |  |  | **Date** |  |

(PI or Lab Supervisor)

1. **HAZARD ASSESSMENT**

Use the hierarchy of controls to document the hazards and the corresponding control measure(s) involved in each step of the procedure.

Consider *elimination or substitution* of hazards, if possible.

***Engineering Control(s):*** items used to isolate the hazard from the user (i.e. fume hood, biosafety cabinet).

***Administrative Control(s****):* policies/programs to limit the exposure to the hazard (i.e. authorizations, designated areas, time restrictions, training).

***Required PPE***: indicate PPE including specific material requirements if applicable (i.e. flame resistant lab coat, type of respirator or cartridge).

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| **Task** | **Hazard** | **Engineering Control(s)** | **Administrative Control(s)** | **Required PPE** |
| Working with Quant-iT dsDNA HS reagent | Flammable substance | Do not work with near ignition source | Training | Nitrile gloves, lab coat, safety glasses |
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1. **TRAINING RECORD**

Use the following table to record the training associated with this Standard Operating Procedure.

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| --- | --- | --- | --- |
| **Print Name** | **Signature** | **Trained By** | **Date** |
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