Preparation of Reagents for quantitative Polymerase Chain Reaction (qPCR)

1. SCOPE AND APPLICATION

1.1. Phosphate buffered saline (PBS) is used during sample preparation for quantitative polymerase chain reaction (qPCR) analysis. It is used to ensure the even dispersion of the sample aliquot during filtration, and to rinse the funnels clean of any sample residue. PBS is also used for making method blanks (MB) at the filtration step.

1.2. Bovine Serum Albumin (BSA) Solution is a serum albumin protein added to the Master Mix to help stabilize enzymes and prevent adhesion to pipette tips and reaction wells.

2. EQUIPMENT AND SUPPLIES

2.1. For PBS:

2.1.1. 2 L volumetric flask or graduated cylinders

2.1.2. 6 L Erlenmeyer flasks (1)

2.1.3. 1000 mL square glass bottle (4)

2.1.4. 30-50 mL beakers (3)

2.1.5. Weigh boats

2.1.6. Magnetic stir bar

2.1.7. Aluminum foil

2.1.8. Diack control ampule

2.1.9. Mettler Toledo MS303S balance (S/N B247544008)

2.1.9.1.Calibrate and use per SOP PCR-2.1

2.1.10. Magnetic stirrer

2.1.11. Accumet Excel XL25 pH meter (S/N XL94005930)

2.1.11.1. Calibrate and use per SOP MB-03.4

2.1.12. Steris AMSCO Model #3021 (S/N RG11420101) autoclave

2.1.12.1. Calibrate and use per SOP TA-06.18.

2.1.13. Thelco Model #51028 Incubator (S/N 41784672), maintained at 35 ± 0.5 °C

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2.2. For BSA:

2.2.1. 15 mL centrifuge tube

2.2.2. Weigh boats

2.2.3. Mettler Toledo MS303S Balance (S/N B247544008)

2.2.3.1.Calibrate and use per SOP PCR-2.1.

3. REAGENTS AND STANDARDS

3.1. For PBS:

3.1.1. Sodium Phosphate Monobasic (NaH_2PO_4)

3.1.2. Disodium Phosphate (Na_2HPO_4)

3.1.3. Sodium chloride (NaCl)

3.1.4. Deionized (DI) water

3.1.5. Sodium hydroxide to adjust the pH.

3.1.6. *E. coli* (ATCC 11775; see SOP MB-03.1 for culture preparation)

3.1.7. TSB2x (for quality control check) - Prepare per SOP MB-02.33.

3.2. For BSA:

3.2.1. Bovine Serum Albumin, fraction V powder

3.2.2. PCR grade water - Autoclave ultrapure water for 15 min. Record in LIMS Autoclave Log.

4. PBS Preparation:

4.1. Using a 2 L volumetric flask or a graduated cylinder, fill a 6 L Erlenmeyer flask with 4 L of DI water in 2L increments. 2L before reagents are added and 2L after to ensure reagents are completely washed off sides.

4.2. Weigh 2.32 g of NaH_2PO_4, 10.0 g of Na_2HPO_4, and 34.0 g of NaCl on separate weigh boats and add to flask. Stir the PBS on the magnetic stirrer using a magnetic stir bar until the reagents are completely dissolved. Cover the flask with aluminum foil. Check the pH of PBS and adjust to 7.4±0.2 with Sodium hydroxide.

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4.3. Hang a Diack control ampule in one of the flasks by the string so that the Diack ampule is partially or completely submerged in the water. Use the foil and autoclave tape to secure the string.

4.4. Autoclave for 30 minutes at 121˚C and 15 psi on wet cycle.

4.5. Immediately after removing the flasks from the autoclave, pour the contents of flask into four 1000 mL square glass bottles. Pour a small amount (3-5 mL) into a separate container to recheck the pH.

4.6. Record preparation in Laboratory Information Management System (LIMS) Standard Preparation Tracker module (**Laboratory/Technical/Standard Preparation Tracker)**. Record the final pH in Standard Prep Tracker. Print labels for the PBS and attach to the bottles when cool. See Section 7 for details.

4.7. Record autoclave use in LIMS AUTOCLAVE_LOG logbook (**Laboratory/Technical/Laboratory Logbook/Autoclave_Log**).

4.8. Store PBS on the shelf at the qPCR filter station for up to 6 months.

5. BSA Preparation:

5.1. Dissolve in PCR grade water to a 2 mg/mL solution before use. Add 20 mg of BSA powder to 10 mL of PCR grade water. Mix thoroughly.

5.2. Store at 4°C for a maximum of 3 months. Record in LIMS Standard Prep Tracker as a prepared reagent (**Laboratory/Technical/Standard Preparation Tracker)**.

6. QUALITY CONTROL

6.1. PBS QC:

6.1.1. Before use, run a quality control check on the PBS Using three 30 mL -50 mL beakers. In 2 beakers make a 1:1 (approx. 25 mL: 25 mL) TSB2x and PBS solution. Add *E. coli* to one of the beakers. This is the positive control. The other beaker is the negative control. To check media sterility, add only TSB2x (~25 mL) to a third beaker. Incubate the three beakers at 35˚C ± 0.5˚C for 24 hours.

6.1.1.1. After incubation, if growth occurs (turbidity) in the negative control, repeat the quality control check. If the repeat test fails, discard the prepared rinse water and make a new batch of PBS.

6.1.1.2. If growth occurs in the TSB2x only beaker, repeat the test with new media.

6.1.2. Record the QC check(s) in LIMS *Standard Preparation Tracker*. Use the **Verify Standard** button to document PBS QC results. In the "Comments" box, copy and paste the comment **"TSB + Rinse = No Turbidity; TSB + Rinse + *E.coli* # = Turbidity; TSB only = No Turbidity, TSB Batch #, Incubated at 35° C for 24**

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hours. Add the serial numbers for both the TSB2x and the *E. coli* and QC results as necessary.

6.2. Autoclave QC

6.2.1. For each batch of PBS made, place a Diack control ampule in one of the flasks as stated in step 4.3.

6.2.1.1. If the Diack has melted after 30 minutes of sterilization, then the autoclave is reaching the proper temperature and pressure for the appropriate amount of time. Check immediately after the flasks are removed from the autoclave. The pellet will not stay melted; it becomes solid as it cools. The shape of the pellet, not a color change, is the indicator.

6.2.1.2. If the Diack has not melted, then discard the batch. Either the autoclave is out of order or the autoclave may have been set to less than 30 minutes.

****Note:** **Do not re-autoclave PBS! This may result in excessive deterioration or decomposition of components.**

7. DOCUMENTATION

7.1. In LIMS select **Laboratory/Technical/Standard Preparation Tracker** from the menu. Choose **BIO-PCR** from the "Group" drop down list.

7.2. Select **Prepared/New Reagent** from the menu. If a similar reagent exists then select **Prepared/Duplicate Reagent** from the menu and choose the reagent to duplicate from the list.

7.3. In the "Prepared Reagent" pop-up window enter the following information:

7.3.1. For PBS: Identity: = PBS; Type: = Working Reagent; Entered by: = Analyst Name; Holding Time: = 180 Days; Prepared = Date/Time of preparation and Analyst; SOP: = PCR-3.0; See Also: = leave blank; Location: = B212; Description = Phosphate Buffered Saline for qPCR; Storage Temperature = Room Temperature; Additional Information: = pH; Solvent Used: = Water; Final Volume: = 1000; Units: = mL;

7.4. Click **Add**. Choose the **Purchased Materials** radio button in the "Select Source:" section. In the "Enter Serial #" pop up window, choose the reagent added to make the preparation being entered from the list, or enter its serial number in the text box. In the "Enter Amount & Units for.." pop up window, enter weight (g) or volume (mL) added (whichever is appropriate) of each reagent added and choose the correct units from the "Added Units:" drop down list. Click **OK**. Do this for each reagent added.

7.5. Click **OK** to save the entry and receive a serial number. Click **Yes** in the "Confirm" pop up window to print labels. In the "Select Label Size & Copies" window, select the 3/4" x 1-1/4" label size in the "Label Size" section. If printing multiple copies, make sure

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that **Use Increment Range** is checked and the "Increment Range:" reflects the number of aliquots made. Click **OK**. Choose the large label printer in the "Print" pop-up window and click **OK**. Place labels on all bottles before storage.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Refer to the Bureau of Laboratories Health and Safety Plan.

8.2. Read MSDS information for reagent handling and disposal.

9. REFERENCES

9.1. EPA Method 1611, October 2012

9.2. Bureau Quality Manual https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops

9.3. Laboratory Health and Safety Plan Health and Safety Plan

9.4. DEP SOP PCR-2.1, *Calibration, Use and Maintenance of Mettler Toledo MS303S Balance*

9.5. DEP SOP MB-02.33, *Preparation of Dehydrated Media*

9.6. DEP SOP MB-03.1, *Maintenance of Bacterial Cultures*

9.7. DEP SOP MB-03.4, *Accumet Excel XL25 pH Meter Calibration and Use*

9.8. DEP SOP TA-06.18, *AMSCO Renaissance Series 3021 Autoclave Calibration and Use*

Appendix of Changes

5/11/2016	Updated to combine 4 flasks into 1 and update incubator. Removed AE buffer preparation which is no longer used.
9/06/2017	Updated 500 mL flask to 1000 mL flask
8/30/2018	Updated 250 mL flask to 30-50 mL beakers

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