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. PBS ensures the even dispersion of the sample during filtration and cleans funnels of any sample residue. PBS serves as the method blank (negative control sample) 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 bottles (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 BB-035

2.1.10. Magnetic stirrer

2.1.11. Accumet Excel XL 25 pH meter (S/N XL94005930)

2.1.11.1. Calibrate and use per SOP MB-03.4

2.1.12. Autoclave, Steris Amsco 250 LS Model 20VS (S/N 031761851)

2.1.13. Heratherm IMH 60 Incubator Model #51028066 (S/N 42511812), maintained at 35 ± 0.5 °C or Fisher Isotemp Model #650F Incubator (S/N 303N0034)

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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 BB-035.

3. REAGENTS AND STANDARDS

3.1. For PBS:

3.1.1. Sodium Phosphate Monobasic (NaH_2PO_4) (Cat.# S369-500)

3.1.2. Sodium Phosphate Dibasic Heptahydrate ($Na_2HPO_4.7H_2O$) (Cat.# S373-500)

3.1.3. Sodium chloride (NaCl) (Cat.# BP358-1)

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. Double strength Tryptic Soy Broth (TSB 2X, for quality control check) - Prepare per SOP MB-02.33.

3.2. For BSA:

3.2.1. Bovine Serum Albumin, fraction V powder (Cat.# BP1600-100)

3.2.2. Molecular Biology grade water – Corning Molecular Biology Grade Water tested to USP Sterile water (Cat.# MT46000CV)

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. Add 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 (Steris cycle #3).

4.5. Immediately after removing the flasks from the autoclave, pour the contents 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 BSA in Molecular Biology grade water to a 2 mg/mL solution before use. Add 20 mg of BSA powder to 10 mL of water. Mix thoroughly.

5.2. Aliquot the BSA solution in 1 mL volume. 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 50 mL beakers. In 2 beakers make a 1:1 (~20 mL: 20 mL) TSB 2X 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 TSB 2X (~20 mL) to a third beaker. Incubate the three beakers at 35 ± 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 PBS and make a new batch.

6.1.1.2. If growth occurs in the TSB 2X 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* # =**

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Turbidity; TSB only = No Turbidity, TSB Batch #, Incubated at 35 ± 0.5˚C for 24 hours. Add the serial numbers for both the TSB 2X and the *E. coli*.

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: = xxx pH; Solvent Used: = Water; Final Volume: = 4000; Units: = mL.

7.3.2. For BSA: Identity: = BSA; Type: = Working Reagent; Entered by: = Analyst Name; Holding Time: = 90 Days; Prepared = Date/Time of preparation and Analyst; SOP: = PCR-3.0; See Also: = leave blank; Location: = B246A; Description = Bovine Serum Albumin 2 mg/mL; Storage Temperature = 4°C; Additional Information: = leave blank; Solvent Used: = Water; Final Volume: = 10000; 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

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"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 that **Use Increment Range** is checked and the "Increment Range:" reflects the number of aliquots made. Click **OK**. Choose the small 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. DEP Laboratory Quality Manual

9.2. Laboratory Health and Safety Plan Health and Safety Plan

9.3. DEP SOP BB-035, *Calibration, Use and Maintenance of Mettler Toledo MS303S Balance*

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

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

9.6. DEP SOP MB-03.4, *Accumet Excel XL25 pH Meter 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
7/31/2020	Updated DEP Laboratory Quality Manual. Added catalog numbers for reagents, added new incubators, and updated SOP #.

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