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. PBS Equipment and Supplies –

Equipment/Supply	Notes
2 L Volumetric Flask or Graduated Cylinder	N/A
6 L Erlenmeyer Flask	N/A
1000 mL Square Glass Bottles	Four bottles per batch
30-50 mL Beakers	Three beakers per batch
Weigh Boats	N/A
Magnetic Stir Bar	N/A
Aluminum Foil	N/A
Diack Control Ampule	N/A
Mettler Toledo MS403TS/00 Balance	S/N B815673069; Calibrate and use per SOP BB-
Magnetic Stirrer	N/A
Oakton PC2700	S/N CP354757; Calibrate and use per SOP MB-3.5
Autoclave, Steris Amsco 250 LS Model 20 VS	S/N 031761851
Heratherm IMH 60 Incubator Model #51028066	S/N 42511812; Maintained at 35 ± 0.5°C
Fisher Isotemp Model #650F Incubator	S/N 303N0034
Autoclave Tape	N/A

2.2. BSA Equipment and Supplies –

Equipment/Supply	Notes
15 mL Centrifuge Tube	N/A
Weigh Boats	N/A
Mettler Toledo MS403TS/00 Balance	S/N B815673069; Calibrate and use per SOP BB-
Refrigerator, Thermo Scientific Model 3751-DB	S/N 0121179101121219; Maintained at 4°C
1.5 mL Microcentrifuge Tubes	Ten tubes per batch

3. REAGENTS AND STANDARDS

3.1. PBS Reagents and Standards –

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Reagent/Standard	Notes
Sodium Phosphate Monobasic (NaH_2PO_4)	Catalog Number: S369-500
Sodium Phosphate Dibasic Heptahydrate ($Na_2HPO_4*7H_2O$)	Catalog Number: S373-500
Sodium Chloride (NaCl)	Catalog Number: BP358-1
Deionized (DI) Water	N/A
Sodium Hydroxide Pellets (NaOH)	To adjust the pH
E. Coli (Working *E. Coli*, ATCC 11775) or equivalent	See SOP MB-03.1 for culture preparation
Double Strength Tryptic Soy Broth (TSB 2X)	For quality control check. Prepare per SOP MB-02.33

3.2. BSA Reagents and Standards –

Reagent/Standard	Notes
Bovine Serum Albumin, Fraction V Powder	Catalog Number: BP1600-100
Molecular Biology Grade Water	Corning Molecular Biology Grade Water tested to USP Sterile Water. Catalog Number: MT46000CV

4. PBS PREPARATION

4.1. Preparing the Phosphate-buffered Saline (PBS) –

Sodium Phosphate Monobasic (NaH_2PO_4)	2.32 g
Sodium Phosphate Dibasic Heptahydrate (Na_2HPO_4)	10.0 g
Sodium Chloride (NaCl)	34.0 g
DI Water	to 4 L
Sodium Hydroxide (NaOH)	to pH 7.4 ± 0.2

4.1.1 Using a 2 L volumetric flask or a graduated cylinder, add 2 L of DI water to a 6 L Erlenmeyer flask.

4.1.2 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.

4.1.3 Add another 2 L of DI water using the 2 L volumetric flask or graduated cylinder. Make sure that reagents are completely rinsed down the sides of the flask.

4.1.4. Add a stir bar and then cover the flask with aluminum foil. Stir the PBS on the magnetic stirrer until the reagents are completely dissolved.

4.1.5. Check the pH of PBS and adjust to 7.4 ± 0.2 with NaOH pellets. Two average sized pellets are typically enough for pH adjustment. Ensure the NaOH is fully dissolved before measuring pH.

4.2. Autoclaving Procedure – Hang a Diack control ampule by a string in the flask so that the wax of the Diack ampule is completely submerged in the water. Use the foil and autoclave tape to secure the string. Autoclave for 30 minutes at 121°C and 15 psi on wet cycle (Steris cycle #3). Record autoclave use in the appropriate autoclave logbook.

NOTE: The Diack control ampule has been discontinued. If a Diack control ampule is not available, autoclave tape may be used instead.

4.3. Immediately after removing the flasks from the autoclave, pour the contents into four 1000 mL square glass bottles. Pour a small amount (approx. 3-5 mL) into a

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separate container to recheck the pH. The water must be room temperature to check the pH.

4.4. Obtain two sterile 100 mL beakers. Pour approx. 25 mL of the PBS into each beaker and cover tightly with the foil lid. Allow the liquid to cool before proceeding to Section 6.1. for QC.

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

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

5. BSA PREPARATION

5.1. Preparing the BSA –

Bovine Serum Albumin	20 mg
Molecular Biology Grade Water	10 mL

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 in a 15 mL centrifuge tube. Mix thoroughly.

Aliquot 1 mL of the BSA solution into sterilized 1.5 mL tubes. 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**). Print labels for the BSA and attach to the microcentrifuge tubes before storing. See Section 7 for details.

6. QUALITY CONTROL

6.1. PBS QC –

6.1.1. Before use, run a quality control check on the PBS poured in Section 4.4. Make a 1:1 solution by adding approximately 25 mL of TSB double strength (2X) to the PBS solution in the beakers. 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. Label each beaker accordingly. Write date and time on each beaker. Incubate the three beakers at 35 ± 0.5°C for 24 hours.

6.1.1.1. If growth occurs (i.e. the solution appears turbid) in the negative control after incubation, discuss with supervisor and repeat the quality control check. If the repeat test fails, discard the prepared PBS and make a new batch.

NOTE: TSB is a non-selective media and is easily contaminated. Ensure use of aseptic technique when handling the media. QC Test failures may be due to improper technique and not necessarily contaminated PBS.

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6.1.1.2. If growth occurs in the PBS + TSB 2X and/or TSB 2X only beaker, repeat the test with new media. If growth occurs again in PBS +TSB 2X beaker, discard the PBS batch and remake.

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* S/N = Turbidity; TSB only = No Turbidity, TSB Batch S/N, Incubated at 35 ± 0.5˚C for 24 hours**." Add the serial numbers for both the TSB 2X and the *E. coli*. List the final pH check of the PBS.

6.2. Autoclave QC –

6.2.1. For each batch of PBS made, place a Diack control ampule in the flask as stated in Section 4.2.

6.2.1.1. If the Diack has melted after 30 minutes of sterilization, then the autoclave is reaching the proper temperature 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.

6.2.2. If a Diack control is unavailable, autoclave tape may be used instead.

6.2.2.1. After the flask is removed, check for the color change on the tape. If no color change is observed, then discard the batch.

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, 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 Name
SOP	PCR-3.0
See Also	Leave Blank
Location	B212
Description	Phosphate Buffered Saline for qPCR
Storage Temperature	Room Temperature

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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 Name
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	10
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 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 Laboratory 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

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9.3. DEP SOP BB-035, *Calibration, Use and Maintenance of Mettler Toledo MS403TS/00 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-03.5, *Oakton PC2700 pH and Conductivity Meter Calibration and Use*

Appendix of Changes

05/11/2016	Updated to combine 4 flasks into 1 and update incubator. Removed AE buffer preparation which is no longer used.
09/06/2017	Updated 500 mL flask to 1000 mL flask
08/30/2018	Updated 250 mL flask to 30-50 mL beakers
07/31/2020	Updated DEP Laboratory Quality Manual. Added catalog numbers for reagents, added new incubators, and updated SOP #.
06/21/2021	Updated Mettler Toledo Balance ID and S/N.
06/17/2022	Updated Equipment, Documentation, and References
06/01/2023	Reformatted SOP and added tables to Sections 2, 3, and 7. Added autoclave tape as an acceptable autoclave QC check in case of Diack unavailability.
06/06/2024	Updated sections 4.2 autoclave logbook documentation and 4.3 pH check. Moved documentation of final pH check from 4.5 to verification section of LIMS in 6.1.2 Clarified procedure for remaking PBS due to QC failure.

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