

ScienceBridge Tech Site Standard Operating Procedure

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| Title: Making phosphate buffer for Enzymes | | |
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| Scope | For use by biotechnology students at the ScienceBridge tech sites when making phosphate buffer solution for the ScienceBridge Enzyme kits. | |
| Objective | This SOP sets the safety and procedural specifications for making a 100mM pH 6 stock solution of potassium phosphate buffer. | |
| Materials | | 2 L stock solution (makes ~17 kits) |
| | 1. Monobasic potassium phosphate 2. Dibasic potassium phosphate 3. Distilled water 4. Plastic weigh dishes 5. pH indicator paper 6. Parafilm | 22.682 g 5.807 g 2 L 2 1 strip (~2in) 2 squares (4in ²) |
| Equipment | 1. Gloves (pair) 2. Goggles 3. Lab coat 4. Balance 5. Graduated cylinder (2000mL capacity) 6. 2L bottle with cap, or large carboy | 1 1 1 1 1 1 |
| Supplemental Aids | • | |
| Safety | 1. Always wear gloves, goggles, and lab coat when handling solutions. Avoid getting solid potassium phosphate on your hands or clothing. | |
| Quantity | 2. <i>Make up to 6 batches of 2 L (12L total)</i> | |
| Protocol | 3. Put on your PPE. 4. Turn on your balance. 5. Place one empty plastic weigh dish on the balance. 6. Press the "tare" or "zero" button on the balance. 7. Use the same plastic weigh dish to measure 22.682g of monobasic potassium phosphate on the balance. 8. Carefully pour the measured monobasic potassium phosphate into the 2 L graduated cylinder. Use a distilled water bottle if some powder is left in the weigh dish. 9. Place a new clean, empty plastic weigh dish on the balance. 10. Press the "tare" or "zero" button on the balance. 11. Use the same plastic weigh dish to measure 5.807g of dibasic potassium phosphate on the balance. | |

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| | <p>12. Carefully pour the measured dibasic potassium phosphate into the same 2L graduated cylinder. Use a distilled water bottle if some powder is left in the weigh dish.</p> <p>13. Carefully fill the graduated cylinder with both the dibasic and monobasic potassium phosphates with about 1.8 L of distilled water.</p> <p>14. Cover the mouth of the graduated cylinder with parafilm. Invert to mix, until the potassium phosphate is completely dissolved. (Check the swirls at the bottom of the graduated cylinder.)</p> <p>15. QC: Test the pH of your phosphate buffer with the pH indicator paper. The pH should be 6.</p> <p>16. Add distilled water until you have a total volume of 2.0 L. Reseal with parafilm and invert to mix.</p> <p>17. Transfer the finished phosphate buffer into a storage container. Be sure to close the bottle or carboy securely.</p> <p>23. Label the bottle or carboy 100mM phosphate buffer, pH=6, with your initials and the date.</p> |
| Documentation | <p>Record who worked on it and how much was supposed to be completed as well as how much was actually completed. Explain any differences in the solutions and aliquots daily log.</p> <p>Additional Protocols: Correcting the pH of buffer solutions</p> |
| Storage | <p>All Supplies should be stored in the solutions and aliquots Cabinet Product Storage</p> <ul style="list-style-type: none"> Store finished and QC'd solutions in the 100 mM, pH 6 phosphate buffer carboy in the chemical storage room. <p>Supplies Storage Locations</p> <ul style="list-style-type: none"> Monobasic and dibasic phosphate buffer is stored in the chemical storage cabinet |
| Quality Control | <p>Fails QC:</p> <ul style="list-style-type: none"> If the pH is off by 1 pH unit (5-7), refer to the SOP "Correcting the pH of buffer solutions." If the pH is off by more than 1 pH unit, you should re-make the buffer solution. |
| When | Make whenever the 100mM phosphate buffer carboy is less than ½ full. |
| Tech Site Group/Kit | Solutions and Aliquots; Enzymes |