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| <p>Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i></p> | <p>HPV Serology Laboratory Standard Operating Procedure</p> | |
| <p>Reagent Preparation for the HPV Serology Laboratory</p> | | |
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Released by/Date Effective:

| Author Name | Title | Signature/Date |
|-----------------|-----------------------|----------------|
| Casper Alabanza | Research Associate II | |

| Approver Name | Title | Signature/Date |
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1. PURPOSE

- 1.1. The purpose of this procedure is to describe the preparation of Reagents, Buffers, and Solutions used in the HPV Serology Laboratory.

2. SCOPE

- 2.1. This procedure applies to the HPV Serology Laboratory located at the Advanced Technology Research Facility, Room C2007.

3. REFERENCES

- 3.1. HSL_GL_006.01: Reagent Preparation Form
- 3.2. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility
- 3.3. HSL_GL_002: Equipment Qualification and Calibration in the HPV Serology Laboratory
- 3.4. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory
- 3.5. HSL_GL_004: Laboratory Notebook Control and Use for the HPV Serology Laboratory
- 3.6. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory
- 3.7. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory
- 3.8. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures
- 3.9. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.
- 4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.
- 4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. REAGENTS, CHEMICALS AND EQUIPMENT

Note: Refer to individual reagent preparation for information relating to the required chemicals and ordering information. Substitutions are allowed if equivalency is indicated.

- 5.1. BSC II

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- 5.2. Analytical Balance
- 5.3. Precision Balance
- 5.4. pH Meter
- 5.5. Refrigerator, 2-8°C
- 5.6. Freezer, -80°C
- 5.7. Freezer, -20 °C
- 5.8. Milli-Q Integral 3 Water System
- 5.9. Serological Pipettes

6. HEALTH AND SAFETY CONSIDERATIONS

- 6.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, closed-toe shoes, safety glasses, and non-latex gloves.
- 6.2. Refer to the respective SDS when working with any chemicals.
- 6.3. Refer to “HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility” regarding waste disposal processes at the ATRF.

7. DEFINITIONS

| Term | Definition |
|---------------|---|
| BSC II | Biological Safety Cabinet Level II |
| Cat # | Catalog number |
| FME | Facilities, Maintenance and Engineering |
| HPV | Human Papillomavirus |
| HSL | HPV Serology Laboratory |
| QS | Quantity Sufficient |
| SDS | Safety Data Sheets |
| SOP | Standard Operating Procedure |
| Type I Water | Ultrapure/Reagent Grade/Critical applications |
| Type II water | Pure/Analytical Grade, used for standard applications |

8. REAGENT PREPARATION GUIDELINES

- 8.1. Each reagent recipe in this SOP will begin on its own page, with its own Section Number.
- 8.2. Each reagent is created using HSL_GL_006.01: Reagent Preparation Form, and requires review by another trained analyst, Scientific Manager or designee prior to assay review.

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8.2.1. It is preferred that the reagent be reviewed prior to use but given the nature of the laboratory, that may not always be possible.

Note: If a reagent is prepared incorrectly but used in an experiment, it may invalidate the experiment. If that occurs, notation is required on the experiment and an investigation may be required.

8.3. Record equipment used in the reagent preparation on page 2 of HSL_GL_006.01: Reagent Preparation Form.

Note: When using a balance, it is recommended to weigh one decimal more than referenced in the reagent's recipe, to ensure rounding and final amounts are accurate.

8.4. Each reagent will be assigned a lot number, based on the QA Assigned Logbook Number and Page Number, where the solution was prepared.

Note: The Lot Number will be the Logbook number plus the first page of the reagent of the QA Assigned page number on HSL_GL_006.01 (e.g. Logbook # = EQ2017099, Reagent recorded on pages 015 and 016. Lot # = EQ2017009015).

8.5. All prepared reagents need to be labelled with Reagent Name, Lot Number, Expiration Date, Storage Conditions, Analyst and Preparation Date. See Attachment 1: Reagent Label Example, for general layout of label.

8.6. Reagents may be scaled up or down in total volume, depending on the need of the laboratory. Check all calculations prior to use to ensure reagent has been made properly.

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10. 5X WASH BUFFER

| Chemical | Supplier/ Cat # (or equivalent) | Amount |
|---|------------------------------------|--------------|
| Sodium Chloride (NaCl) | VWR, Cat # EM1.06404.5000 | 404 ± 0.4 g |
| Potassium Phosphate, Monobasic (KH ₂ PO ₄) | VWR, Cat# PX1565-1 | 4± 0.2 g |
| Sodium phosphate dibasic anhydrous (Na ₂ HPO ₄) | VWR, Cat #97061-584 | 18.34± 0.2 g |
| Tween- 20 | VWR, Cat #EM-9480 | 10 mL |
| Type I water | Milli-Q Integral 3 Water System | QS 4 L |

- 10.1. Weigh out 404± 0.4 g of Sodium Chloride and add it to a container.
- 10.2. Weigh out 4± 0.2 g of Potassium Phosphate, Monobasic and add it to the container.
- 10.3. Weigh out 18.34± 0.2 g of Sodium phosphate dibasic anhydrous and add it to a container.
- 10.4. Add approximately 3 L of Type I water to dissolve the chemicals.
- 10.5. Once chemicals are dissolved, add 10 mL of Tween-20 using a serological pipette. Rinse pipette well in the solution.
- 10.6. Once all chemicals are dissolved, QS reagent to 4 L using Type I water.
- 10.7. Reagent expires 2 months from date of preparation and must be stored at 2-8°C.

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11. 1X WASH BUFFER

| Chemical | Source / Cat # (or equivalent) | Amount |
|----------------|------------------------------------|--------|
| 5X Wash Buffer | HSL_GL_005, Section 10 | 1 L |
| Type I water | Milli-Q Integral 3 Water System | 4 L |

11.1. Mix 1 L of 5X Wash Buffer with 4 L of Type I water.

11.2. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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12. HPV ELISA COATING BUFFER

| Chemical | Source / Cat # | Amount |
|--|---------------------------------|--------|
| Proclin 300 | Sigma-Aldrich, Cat # 48914-U | 2 mL |
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 998 mL |

- 12.1. Combine 2 mL of Proclin 300 with 998 mL PBS in a 1 L container.
- 12.2. Swirl until mixed.
- 12.3. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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13. DPBS AND 0.2% TWEEN 20 (DPBS_0.2T)

| Chemical | Source / Cat # (or equivalent) | Amount |
|--|--------------------------------|--------|
| Tween- 20 | VWR, Cat # EM-9480 | 2 mL |
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 998 mL |

- 13.1. Add 998 mL of DPBS to a reagent container.
- 13.2. Add 2 mL of Tween-20 using a serological pipette then rinse pipette until a majority of Tween-20 has been removed from the tip.
- 13.3. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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14. 2N H₂SO₄

| Chemical | Source / Cat # (or equivalent) | Amount |
|--|------------------------------------|----------|
| Sulfuric Acid (1N H ₂ SO ₄) | VWR, Cat # JT4700-1 | 1 ampule |
| Type I water | Milli-Q Integral 3 Water System | 500 mL |

- 14.1. Measure ~400 mL of Type I water and put it into a Glass Volumetric Flask.
- 14.2. Carefully break open one concentrated sulfuric acid ampule and add it to the Glass Volumetric Flask.
- 14.3. QS volume of the solution to 500 mL with Type I water.
- 14.4. Reagent expires 6 months from date of preparation and must be stored at room temperature.

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15. 0.36N H₂SO₄

| Chemical | Source / Cat # (or equivalent) | Amount |
|--|------------------------------------|--------|
| HPV ELISA Stop Solution- Stock 2N H ₂ SO ₄ | Section 14, HSL_GL_006 | 180 mL |
| Type I water | Milli-Q Integral 3 Water System | 820 mL |

- 15.1. Add 820 mL of Type I water to a 1L container.
- 15.2. Add 180 mL of 2N H₂SO₄ to the container.
- 15.3. Reagent expires 1 month from date of preparation and must be stored at room temperature.

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16. TARTRAZINE SOLUTION

| Chemical | Source / Cat # (or equivalent) | Amount |
|--|--------------------------------|--------------|
| Tartrazine | Sigma-Aldrich, Cat # T0388 | 224 ± 0.4 mg |
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 1920 mL |

- 16.1. Add 1920 mL of DPBS to a storage container.
- 16.2. Measure 224 mg of Tartrazine and add it to the DPBS.
- 16.3. Shake vigorously until all powder has been dissolved.
- 16.4. Reagent expires 1 year from date of preparation and must be stored at room temperature in an amber container (protect from light).

Note: Discard reagent if the Optical Density value drops below 2.20.

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17. 293TT THAWING MEDIA (293TT TM)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|---|--------------------------------|--------|
| Dulbecco's Modified Eagle's Medium (DMEM) | Gibco, Cat# 11965-126 | 78 mL |
| Heat inactivated fetal bovine serum (FBS) | Hyclone, Cat# SH30070.03HI | 20 mL |
| MEM Non-Essential Amino Acids | Gibco, Cat# 11140-050 | 1 mL |
| Glutamax I | Gibco, Cat# 35050-061 | 1 mL |
| 0.2 µm PES filter (250 mL) | VWR, Cat # 73520-988 | 1 unit |

- 17.1. Combine 78 mL of DMEM, 20 mL of FBS, 1 mL of MEM non-essential amino acids and 1 mL Glutamax I.
- 17.2. Filter using a 0.2 µm PES filter.
- 17.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C.

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18. 293TT MAINTENANCE MEDIA (293TT MM)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount | |
|---|--------------------------------|---------|---------|
| Dulbecco's Modified Eagle's Medium (DMEM) | Gibco, Cat# 11965-126 | 87.2 mL | 500 mL |
| Heat inactivated fetal bovine serum (FBS) | Hyclone, Cat# SH30070.03HI | 10 mL | 57.5 mL |
| MEM Non-Essential Amino Acids | Gibco, Cat# 11140-050 | 1 mL | 5.7 mL |
| Glutamax I | Gibco, Cat# 35050-061 | 1 mL | 5.7 mL |
| Hygromycin B | Gibco, Cat# 10687-010 | 0.8 mL | 4.6 mL |
| 0.2µm PES filter | VWR, Cat # 73520-988 | 1 unit | 1 unit |

- 18.1. Combine DMEM, FBS, MEM non-essential amino acids, Glutamax I, and Hygromycin B.
- 18.2. Filter using 0.2 µm PES filter.
- 18.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C.

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19. 293TT FREEZING MEDIA (293TT FM)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|---|--------------------------------|---------|
| Heat inactivated fetal bovine serum (FBS) | Hyclone, Cat# SH30070.03HI | 41.0 mL |
| Dimethyl sulfoxide (DMSO) | Thomas Sci., Cat # C999K06 | 9.0 mL |
| 0.2µm PES filter | VWR, Cat # 73520-988 | 1 unit |

- 19.1. Combine 41 mL of FBS and 9 mL of DMSO.
- 19.2. Filter using 0.2µm PES filter.
- 19.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C.

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20. 70% ETHANOL

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|---------------------|--|--------|
| 200 Proof Ethanol | Sigma, Cat # E-7023-500ml | 28 mL |
| Distilled Water | Life Technologies, Cat # 15-230-001 | 12 mL |
| 50 mL Conical Tube | Warehouse, Cat # 66401493 | 1 tube |

- 20.1. Combine 28 mL of 200 Proof Ethanol and 12 mL of Distilled water in a 50 mL conical tube.
- 20.2. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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21. 293TT VLP/PSV TRANSFECTION CELL CULTURE MEDIA (DMEM-10A)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount | |
|---|--------------------------------|---------|----------|
| Dulbecco's Modified Eagle's Medium (DMEM) | Gibco, Cat# 11965-126 | 87.2 mL | 174.4 mL |
| Heat inactivated fetal bovine serum (FBS) | Hyclone, Cat# SH30070.03HI | 10 mL | 20 mL |
| MEM Non-Essential Amino Acids | Gibco, Cat# 11140-050 | 1 mL | 2 mL |
| Glutamax I | Gibco, Cat# 35050-061 | 1 mL | 2 mL |
| 0.2µm PES filter | VWR, Cat # 73520-988 | 1 unit | 1 unit |

- 21.1. Combine DMEM, FBS, MEM non-essential amino acids, and Glutamax I.
- 21.2. Filter using 0.2µm PES filter.
- 21.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C

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22. DPBS-MGCL2 10MM A/A (DPBS_MGCL_AA)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|--------------------------------|--------|
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 98 mL |
| 1M Magnesium Chloride (MgCl ₂) | KD Medical, Cat# PMS-0630 | 1 mL |
| Antibiotic-Antimycotic (100x) | Gibco, Cat# 15240-062 | 1 mL |
| 0.2µm PES filter | VWR, Cat # 73520-988 | 1 unit |

- 22.1. Combine 98 mL DPBS, 1 M MgCl₂, and 1 mL Antibiotic/Antimycotic (A/A).
- 22.2. Filter using a 0.2 µm PES filter.
- 22.3. Reagent must be stored at 2-8°C and is good for up to one month after preparation.

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23. 10% BRIJ58

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|--------------------------------|--------------|
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | QS to 100 mL |
| Brij58 | Sigma, Cat# P5884 | 10 ± 0.4 g |

- 23.1. Dissolve 10 ± 0.4 g in 80 mL DPBS overnight.
- 23.2. Once Brij58 has been dissolved, QS up to 100 mL in DPBS.
- 23.3. Detergent must be stored at 2-8°C and is good for up to two months after preparation.

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24. DPBS/0.8M SALT BUFFER (DPBS_0.8M)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|-------------------------------------|----------|
| Type I Water | Milli-Q Integral 3 Water System | 153.5 mL |
| 10X Dulbecco's phosphate-buffered saline | Fisher Scientific, Cat # 14-200-075 | 20 mL |
| 5M Sodium Chloride (NaCl) | KD Medical, Cat# RGF-3270 | 25 mL |
| 1M Calcium Chloride (CaCl ₂) | KD Medical, Cat# PMS-0614 | 180 µL |
| 1M Magnesium Chloride (MgCl ₂) | KD Medical, Cat# PMS-0630 | 100 µL |
| 1M Potassium Chloride (KCl) | KD Medical, Cat# PMS-0642 | 420 µL |
| 0.2 µm PES filter | VWR, Cat # 73520-988 | 1 unit |

- 24.1. Combine 153.5 mL Type 1 Water, 20 mL 10X DPBS, 25 mL 5M NaCl, 180µL 1M CaCl₂, 100µL 1M MgCl₂, and 420µL 1M KCl.
- 24.2. Filter using a 0.2 µm PES filter.
- 24.3. Reagent must be stored at 2-8°C and is good for up to two months after preparation.

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25. 46% OPTIPREP

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|--|--------|
| 60% OptiPrep | Sigma, Cat # D1556-250ml | 77 mL |
| 10X Dulbecco's phosphate-buffered saline | Fisher Scientific, Cat # 14-200-075 | 10 mL |
| 5M Sodium Chloride (NaCl) | KD Medical, Cat# RGF-3270 | 13 mL |
| 1M Calcium Chloride (CaCl ₂) | KD Medical, Cat# PMS-0614 | 92 µL |
| 1M Magnesium Chloride (MgCl ₂) | KD Medical, Cat# PMS-0630 | 52 µL |
| 1M Potassium Chloride (KCl) | KD Medical, Cat# PMS-0642 | 200 µL |

- 25.1. Combine 77 mL 60% OptiPrep, 10 mL 10X PBS, 13 mL 5M NaCl, 92 µL 1M CaCl₂, 52 µL 1M MgCl₂, and 0.2 mL 1M KCl in 150 mL sterile bottle.
- 25.2. Protect from light.
- 25.3. Gradient must be stored at room temperature and is good for up to two months after preparation.

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26. 27% OPTIPREP

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|-----------------------------------|--------------------------------|---------|
| 46% OptiPrep | HSL_GL_006, Section 25 | 26.4 mL |
| DPBS/0.8M SALT BUFFER (DPBS_0.8M) | HSL_GL_006, Section 24 | 18.6 mL |

- 26.1. Combine 26.4 mL of 46% OptiPrep with 18.6 mL of DPBS/0.8M SALT BUFFER (DPBS_0.8M).
- 26.2. Protect from light.
- 26.3. Gradient must be stored at room temperature and is good for up to two months after preparation.

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27. 33% OPTIPREP

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|--------------------------------|---------|
| 46% OptiPrep | HSL_GL_006, Section 25 | 32.2 mL |
| Dulbecco's phosphate-buffered saline (DPBS)/0.8M Salt Buffer | HSL_GL_006, Section 24 | 12.8 mL |

- 27.1. Combine 32.2 mL of 46% OptiPrep with 12.8 mL of DPBS/0.8M SALT BUFFER (DPBS_0.8M).
- 27.2. Protect from light.
- 27.3. Gradient must be stored at room temperature and is good for up to two months after preparation.

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28. 39% OPTIPREP

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|-----------------------------------|--------------------------------|--------|
| 46% OptiPrep | HSL_GL_006, Section 25 | 38 mL |
| DPBS/0.8M Salt Buffer (DPBS_0.8M) | HSL_GL_006, Section 24 | 6.8 mL |

- 28.1. Combine 38 mL of 46% OptiPrep with 6.8 mL of DPBS/0.8M SALT BUFFER (DPBS_0.8M).
- 28.2. Protect from light.
- 28.3. Gradient must be stored at room temperature and is good for up to two months after preparation.

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29. 293TT PSEUDOVIRION BASED NEUTRALIZATION ASSAY MEDIA (PBNA_M)

| Chemical/Consumable | Source / Cat # | Amount for 200 mL | Amount for 500 mL |
|---|--------------------------------|-------------------------|-------------------------|
| Phenol red-free Dulbecco's Modified Eagle Medium (DMEM) | Invitrogen, Cat# 21063-029 | 172 mL | 430 mL |
| Heat inactivated fetal bovine serum (FBS) | Hyclone, Cat# SH30070.03HI | 20 mL | 50 mL |
| Glutamax (100x) | Invitrogen, Cat# 35-050-061 | 2 mL | 5 mL |
| Antibiotic-Antimycotic | Invitrogen, Cat# 15240-062 | 2 mL | 5 mL |
| HEPES (1M) | Invitrogen, Cat# 15630-080 | 2 mL | 5 mL |
| MEM, non-essential amino acids (100x) | Invitrogen, Cat# 11-140-050 | 2 mL | 5 mL |
| 0.2 µm PES filter | VWR, Cat # 73520-988 | 1 unit | |

- 29.1. Combine Phenol red-free DMEM, FBS, Glutamax, Anti-Anti, HEPES, MEM NEAA together into the top of a filter unit. Filter media using a 0.2µm PES filter.
- 29.2. If preparing 500 mL, store in two separate aliquots to prevent accidental contamination.
- 29.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C.

Note: New stock media should be made for each neutralization assay and not shared between experiments or cell maintenance.

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30. 1M AMMONIUM SULFATE

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|------------------------------------|-------------|
| Ammonium Sulfate | Sigma, Cat# A4418 | 6.6 ± 0.1 g |
| Type I Water | Milli-Q Integral 3 Water System | QS to 50 mL |
| Millex 33 mm, Durpre PVDF 0.22 µm filter | Thomas Scientific, Cat# 1211K48 | 1 Unit |
| 60 mL syringe | Warehouse, Cat# 66301460 | 1 Syringe |
| 60 mL PETG Storage Bottle | Thomas Scientific, Cat# 1720N26 | 1 Bottle |
| Sodium Hydroxide | Sigma, Cat# 795429 | As Needed |

- 30.1. Dissolve Ammonium Sulfate into 45 mL of Type I water in a 100 mL beaker.
- 30.2. Adjust pH to 9.0 using sodium hydroxide, then QS to 50 mL with Type I water.
- 30.3. Filter solution using a Millex 33 mm, Durpre PVDF 0.22 µm filter and 60 mL syringe into a 60 mL PETG Storage Bottle.
- 30.4. Cover bottle with aluminum foil, and store at 2-8°C for up to 2 months.

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31. COUPLING ASSAY BUFFER

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|-------------------------------------|----------|
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 100 mL |
| BSA | Sigma, Cat # A-2153 | 1±0.03 g |
| 0.2 µm PES filter | Thomas Scientific, Cat # 1234K60 | 1 unit |

- 31.1. Combine 1X DPBS and BSA, mix to dissolve.
- 31.2. Filter using a 0.2µm PES filter.
- 31.3. Solution must be stored at 2-8°C and is good for up to nine months after preparation.

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32. 10% TWEEN 20

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|---|-----------------------------------|--------|
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 90 mL |
| Tween 20 | VWR, Cat # EM-9480 | 10 mL |

- 32.1. Add 90 mL of 1X DPBS to a container.
- 32.2. Add 10 mL of Tween 20 to container using a serological pipette, rinse the pipette well.
- 32.3. Solution must be stored at 2-8°C, protected from light (may use amber bottle or aluminum foil) and is good for up to six months after preparation.

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33. HPV LUMINEX SAMPLE DILUENT

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|-------------------------------------|-----------|
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 500 mL |
| BSA | Sigma, Cat # A2153-10G | 5 ± 0.2 g |
| Proclin 300 | Sigma-Aldrich, Cat # 48914-U | 1 mL |
| Tween 20 | Sigma, Cat # 9480-1L | 1 mL |
| 0.2 µm PES filter | Thomas Scientific, Cat # 1234K58 | 1 unit |

- 33.1. Add 500 mL of 1X DPBS to a container.
- 33.2. Weight out 5 ± 0.2 g of BSA and dissolve in 1X DPBS.
- 33.3. Add 1 mL of Proclin 300. Swirl to mix.
- 33.4. Add 1 mL of Tween 20 to container using a serological pipette, rinse the pipette well. Gently swirl to mix to avoid creating bubbles.
- 33.5. Filter using a 0.2 µm PES filter.
- 33.6. Solution must be stored at 2-8°C and is good for up to one month after preparation.

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34. PEI WITH 5% GLUCOSE (PEI_5%G)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|---|----------------------------------|--------------|
| Polyethylenimine, Linear, MW 25,000 (PEI 25000) | Polysciences, Cat # 23966-1 | 0.3 ± 0.02 g |
| Distilled Water | Gibco, Cat # 15-230-001 | 300 mL |
| D-(+)-Glucose | Sigma, Cat # G7021-1KG | 15 ± 0.5 g |
| 0.2 µm PES filter | Thomas Scientific, Cat # 1234K58 | 1 unit |

- 34.1. Dissolve PEI in Distilled water. Drop pH to 2.4 ± 0.2 with HCl.
- 34.2. Warm mixture on stir plate. Utilize stir bar to maintain mixing for approximately 2 hours. Once PEI has dissolved, allow mixture to reach room temperature then pH solution using NaOH to 7.2 ± 0.1.
- 34.3. Add glucose to solution and mix until dissolved.
- 34.4. Filter using a 0.2 µm PES filter.
- 34.5. Prepare 10 mL aliquots and label. Label per Attachment 1: Reagent Label Example.
- 34.6. Solution must be stored at -15 to -25°C and is good for up to one year after preparation.

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35. DPBS/0.5M SALT BUFFER (DPBS_0.5M)

| Chemical/Consumable | Source / Cat # (or equivalent) | Amount |
|--|--------------------------------|---------|
| 1X Dulbecco's phosphate-buffered saline (DPBS) | Gibco Cat# 14190-136 | 92.5 mL |
| 5M Sodium Chloride (NaCl) | KD Medical, Cat# RGF-3270 | 7.5 mL |
| 0.2 µm PES filter | VWR, Cat # 73520-988 | 1 unit |

- 35.1. Combine 1X DPBS and 5M NaCl then filter using a 0.2 µm PES filter.
- 35.2. Reagent must be stored at 2-8°C and is good for up to two months after preparation.

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36. ATTACHMENTS

36.1. Attachment 1: Reagent Label Example

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Attachment 1: Reagent Label Example

Reagent Preparation Label:

| |
|---|
| <p>Reagent Name: _____</p> <p>Lot Number: _____</p> <p>Expiration Date: _____</p> <p>Storage: _____</p> <p>Analyst/Date: _____</p> <p>Reviewed By/Date: _____</p> |
|---|

Aliquot Label:

| |
|--|
| <p>Reagent Name: _____</p> <p>Lot #: _____</p> <p>Expiration Date: _____</p> <p>Analyst/ Aliquot Date: _____</p> |
|--|

Note: Information based on size of label. “Reagent Name” and “Analyst/ Aliquot Date” do not need to be written, just the information)

Example:

| |
|--|
| <p><i>70% Ethanol</i></p> <p><i>Lot #: 13Apr17-01</i></p> <p><i>Exp: 13Apr18</i></p> <p><i>ABC 13Apr17</i></p> |
|--|

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37. REVISION HISTORY

| Revision Start Date | Version # | Changes | Reasons |
|---------------------|-----------|---|---|
| 30Mar17 | New | Create SOP for the preparation of Reagents, Buffers and Solutions used in HSL. | New SOP. |
| 24Aug17 | 1.0 | Update list of reagents to add new ones. Add options for varied volumes of reagents. | New reagents. Prevent errors when increasing or decreasing prepared volumes. |

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| Reagent Preparation Form | | | |
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| Reagent Name: | | | |
|-------------------------------------|------------|-------------------------|-------------|
| Assigned Reagent Lot Number: | | | |
| Preparation Date: | | Expiration Date: | |
| Storage Conditions: | | | |
| Chemical | Lot Number | Expiration Date | Amount Used |
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| Comments: <div style="border: 1px solid black; height: 100px; width: 100%;"></div> |
| <input type="checkbox"/> N/A |

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|--------------------|
| Prepared By/ Date: |
| Reviewed By/ Date: |

| | | |
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Balance: N/A

| Equipment ID | Calibration Due Date |
|------------------|----------------------|
| Affix Print out: | |

pH Meter: N/A

| | | |
|------------------|-----------|------------------------------------|
| Required pH: | Final pH: | Adjusted with Chemical/Lot/Exp: |
| Equipment ID | | Calibration Due Date |
| Affix Print out: | | |

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| Comments: | <input type="checkbox"/> N/A |
|-----------|------------------------------|

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| Prepared By/ Date: |
| Reviewed By/ Date: |