

**Frederick National Laboratory
for Cancer Research**

sponsored by the National Cancer Institute

Vaccine, Immunity and Cancer Directorate
Standard Operating Procedure

SOP Title: Reagent Preparation

Document ID: 15006

Version

1.0

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Supersedes

New

Effective Date: 18Nov21

Written by:

Printed 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.

2. SCOPE

- 2.1. This procedure applies to the Vaccine, Immunology, and Cancer Directorate (VICD).

3. REFERENCES

- 3.1. 10005: Identification, Control and Use of Laboratory Notebooks
- 3.2. 10010: Lot Number and Test Run Number Assignment for Non-Controlled Issuance Documents
- 3.3. 10023: Good Documentation Practices
- 3.4. 15000: Waste Disposal at the Advanced Technology Research Facility
- 3.5. 15011: Reagent and Chemical Expiry
- 3.6. 26002: Use and Maintenance of the BioTek Plate Washer
- 3.7. 26011: Use and Maintenance of a pH Meter
- 3.8. 26012: Use and Maintenance of an Analytical and Precision Balance
- 3.9. 26016: Operation, Use and Maintenance of the Water Purification Systems
- 3.10. 30000: HPV Neutralization Assay for Titer Determination

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.

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5. DEFINITIONS

- 5.1. QS - Quantity Sufficient
- 5.2. TOC - Total Oxidizable Carbon
- 5.3. Type I Water - Ultrapure/Reagent Grade/critical applications (Resistivity > 18 M Ω -cm and TOC \leq 50 ppb)
- 5.4. Type II Water - Pure/Analytical Grade, used for standard applications (Resistivity > 1 M Ω -cm and TOC \leq 50 ppb)

6. REAGENTS, MATERIALS 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.

- 6.1. Aluminum foil
- 6.2. Analytical Balance
- 6.3. Class II Biosafety Cabinet (BSC)
- 6.4. Freezer, -10 to -30°C
- 6.5. Overhead stirrer
- 6.6. pH Meter
- 6.7. Pipet aid
- 6.8. Precision Balance
- 6.9. Refrigerator, 2-8°C
- 6.10. Stirbars and Stirplate
- 6.11. Storage containers, various
- 6.12. Vortexer
- 6.13. Water Purification Systems Serological Pipettes

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7. HEALTH AND SAFETY CONSIDERATIONS

- 7.1. Proper safety precautions must 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, non-latex gloves, and sharps protective gloves, as needed.
- 7.2. Refer to the respective Safety Data Sheet (SDS) when working with any chemicals.
- 7.3. Refer to "15000: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the Advanced Technology Research Facility (ATRF).
- 7.4. Refer to "EHS-WM-1, Disposal and Minimization of Chemical Waste" and "EHS-WM-2, Biological Waste Handling and Disposal" regarding waste disposal processes at the Ft. Detrick campus.

8. PROCEDURE PRINCIPLES

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- 8.1. Each reagent recipe in this procedure begins on its own page, with its own Section Number. See the reagent recipe for the chemicals needed; chemicals are not listed in section 6 of this procedure.
- 8.2. Each reagent is made using "15006-01: Reagent Preparation Form" except for HPV Plate Coating which uses "15006-02: HPV Plate Coating Form."
- 8.3. Record equipment used in the reagent preparation of 15006-01.
- 8.4. 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.
- 8.5. Each reagent is assigned a lot number per "10010: Lot Number and Test Run Number Assignment for Non-Controlled Issuance Documents".
- 8.6. All prepared reagents are labelled with Reagent Name, Lot Number, Expiration Date, Storage Conditions, Analyst Initials and Preparation Date. See "Attachment 1: Reagent Label Example" for general layout of label.
- 8.7. Use Hydrochloric Acid (HCl) to lower the pH of a reagent and use Sodium Hydroxide (NaOH) to raise the pH of a reagent.
- 8.8. Reagents are scaled up or down in total volume, depending on the need of the laboratory. If making larger volumes of a reagent that requires filtering, the same 0.2 µm PES filter can be used. Change filter when clogged.

Note: Check all calculations prior to use to ensure reagent will be made properly.

- 8.9. All measured volumes have a tolerance limit of $\pm 1\%$.
- 8.10. If a reagent is prepared outside of the tolerance limits established in this SOP, then the reagent must be discarded and not used for clinical sample testing.
- 8.11. Reagents that are not captured in this procedure, or a process specific procedure, can be captured by one of the following:
 - 8.11.1. Recording preparation in a laboratory notebook per "10005: Identification, Control and Use of Laboratory Notebooks." The first page of preparation is used as the page number in the data reference per 10010.
 - 8.11.2. Using form 15006-01.
 - 8.11.2.1. When preparing a reagent that's not included in this SOP, select "Reagent not included in 15006; see attached preparation protocol"

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in the comments section and attach a reagent preparation protocol. The protocol can be a reference document such as a publication, or vendor document, or printed copy authored by analyst.

- 8.11.2.2. Record the reagent lot number on the protocol and initial/date before attaching to 15006-01 per "10023: Good Documentation Practices."

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10. 5X Wash Buffer

Chemical	Supplier/ Cat # (or equivalent)	Amount (4L)	Amount (20L)
Sodium Chloride (NaCl)	VWR, Cat # EM1.06404.5000	404 ± 0.4 g	2,020 ± 2.0 g
Potassium Phosphate, Monobasic (KH ₂ PO ₄)	VWR, Ca t# PX1565-1	4 ± 0.2 g	20 ± 1.0 g
Sodium Phosphate Dibasic Anhydrous (Na ₂ HPO ₄)	VWR, Cat # 97061-584	18.34 ± 0.2 g	91.7 ± 1.0 g
TWEEN® 20 (T20)	VWR, Cat # EM-9480	10 mL	50 mL
Type I Water	Water System, 26016 or equivalent	QS 4 L	QS 20 L

- 10.1. For 4 liters (4L), weigh out 404 ± 0.4 g of Sodium Chloride and add it to an appropriately sized 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 the container.
- 10.4. Add approximately 3 L of Type I water to dissolve the chemicals.

Note: May add stir bar to the container and mix without heat or use an overhead stirrer to mix contents. Chemicals can take approximately 1-2 hours to dissolve.

- 10.5. Once chemicals are dissolved, add 10 mL of TWEEN® 20 using a serological pipette. Rinse pipette well in the solution.
- 10.6. 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	15006, Section 10	1 L
Type I water	Water System, 26016 or equivalent	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 # (or equivalent)	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 DPBS 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 (T20)	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. Rinse pipette well in the solution.
- 13.3. Reagent expires 1 month from date of preparation and must be stored at 2-30°C.

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14. 2N H₂SO₄

Chemical	Source / Cat # (or equivalent)	Amount
Sulfuric Acid (H ₂ SO ₄)	VWR, Cat # JT4700-1	1 ampule
Type I Water	Water System 26016 or equivalent	QS 500 mL

- 14.1. Carefully open one concentrated Sulfuric Acid ampule wearing non-latex gloves and sharps protective gloves and pour into a 500 mL Glass Volumetric Flask.
- 14.2. QS volume of the solution in the Volumetric Flask to 500 mL with Type I Water.
- 14.3. Reagent expires 6 months from date of preparation and must be stored at room temperature in a secondary container, inside a flammable cabinet.

15. 0.36N H₂SO₄

Chemical	Source / Cat # (or equivalent)	Amount
2N Sulfuric Acid (2N H ₂ SO ₄)	Section 14, 15006	180 mL
Type I Water	Water System, 26016 or equivalent	820 mL

16. Add 820 mL of Type I Water to a 1 L container.

- 16.1. Add 180 mL of 2N H₂SO₄ to the container.
- 16.2. Reagent expires 1 month from date of preparation and must be stored at room temperature in a secondary container, inside a flammable cabinet.

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17. 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

- 17.1. Add DPBS to an amber storage container. Typically made in bulk such as 2 liters.
- 17.2. Measure Tartrazine in grams on an analytical scale with QuickLock glass panels (or equivalent) assembled, then add it to the DPBS.
 - 17.2.1. If Tartrazine residue remains on weigh boat, the weigh boat may be rinsed with some DPBS from step 16.1 and then added to the storage container.
- 17.3. Shake vigorously until all powder has been dissolved.
- 17.4. Reagent must be stored in an amber container and protected from light. Select "Protected from light" on 15006-01.
- 17.5. Reagent expires 1 year from date of preparation and must be stored at room temperature.

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18. 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 (NEAA)	Gibco, Cat # 11140-050	1 mL
Glutamax I (Glut)	Gibco, Cat # 35050-061	1 mL
0.2 µm PES filter (250 mL)	VWR, Cat # 73520-988	1 unit

- 18.1. Combine 78 mL of DMEM, 20 mL of FBS, 1 mL of MEM non-essential amino acids, and 1 mL Glutamax I together into the top of a filter unit.

Note: Can scale total volume up or down as needed.

- 18.2. Filter using a 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 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	436 mL
Heat Inactivated Fetal Bovine Serum (FBS)	Hyclone, Cat # SH30070.03HI	10 mL	50 mL
MEM Non-Essential Amino Acids (NEAA)	Gibco, Cat # 11140-050	1 mL	5 mL
Glutamax I (Glut)	Gibco, Cat # 35050-061	1 mL	5 mL
Hygromycin B (Hygro-B)	Gibco, Cat# 10687-010	0.8 mL	4 mL
0.2 µm PES Filter	VWR, Cat # 73520-988	1 unit	1 unit

- 19.1. Combine DMEM, FBS, MEM non-essential amino acids, Glutamax I, and Hygromycin B together into the top of a filter unit.
- 19.2. Filter using 0.2 µm 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. 293TT Freezing Media (293TT FM)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Heat Inactivated Fetal Bovine Serum (FBS)	Hyclone, Cat # SH30070.03HI	41 mL
Dimethyl Sulfoxide (DMSO)	Thomas Sci., Cat # C999K06	9 mL
0.2 µm PES Filter	VWR, Cat # 73520-988	1 unit

- 20.1. Combine 41 mL of FBS and 9 mL of DMSO together into the top of a filter unit.
- 20.2. Filter using 0.2 µm PES filter.
- 20.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C.

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21. 70% Ethanol

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
200 Proof Ethanol (EtOH)	Sigma, Cat # E-7023-500ml	28 mL
Distilled Water (DH ₂ O)	Life Technologies, Cat # 15-230-001	12 mL

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

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22. 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	88 mL	880 mL
Heat Inactivated Fetal Bovine Serum (FBS)	Hyclone, Cat # SH30070.03HI	10 mL	100 mL
MEM Non-Essential Amino Acids (NEAA)	Gibco, Cat # 11140-050	1 mL	10 mL
Glutamax I (Glut)	Gibco, Cat # 35050-061	1 mL	10 mL
0.2 µm PES Filter	VWR, Cat # 73520-988	1 unit	1 unit

- 22.1. Combine DMEM, FBS, MEM non-essential amino acids, and Glutamax I together into the top of a filter unit.
- 22.2. Filter using 0.2 µm PES filter.
- 22.3. Reagent expires 2 weeks from date of preparation and must be stored at 2-8°C.

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23. 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) (A/A)	Gibco, Cat# 15240-062	1 mL
0.2 µm PES Filter	VWR, Cat # 73520-988	1 unit

- 23.1. Combine 98 mL DPBS, 1 M MgCl₂, and 1 mL Antibiotic/Antimycotic (A/A) together into the top of a filter unit.
- 23.2. Filter using a 0.2 µm PES filter.
- 23.3. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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24. 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

- 24.1. Dissolve 10 ± 0.4 g in 80 mL DPBS overnight.
- 24.2. Once Brij58 has been dissolved, QS up to 100 mL in DPBS.
- 24.3. Reagent expires 2 months from date of preparation and must be stored at 2-8°C.

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25. DPBS/0.8M Salt Buffer (DPBS_0.8M)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Type I Water	Water System, 26016 or equivalent	153.5 mL
10X Dulbecco's Phosphate-Buffered Saline (10XDPBS)	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

- 25.1. Combine 153.5 mL Type I Water, 20 mL 10X DPBS, 25 mL 5M NaCl, 180 µL 1M CaCl₂, 100 µL 1M MgCl₂, and 420 µL 1M KCl together into the top of a filter unit.
- 25.2. Filter using a 0.2 µm PES filter.
- 25.3. Reagent expires 2 months from date of preparation and must be stored at 2-8°C.

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26. 46% OptiPrep

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
60% OptiPrep	Sigma, Cat # D1556-250ml	77 mL
10X Dulbecco's Phosphate-Buffered Saline (10XDPBS)	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

- 26.1. Combine 77 mL 60% OptiPrep, 10 mL 10X DPBS, 13 mL 5M NaCl, 92 µL 1M CaCl₂, 52 µL 1M MgCl₂, and 200 µL 1M KCl in 150 mL sterile bottle.
- 26.2. Protect from light. Select "Protected from light" on 15006-01.
- 26.3. Reagent expires 2 months from date of preparation and must be stored at room temperature.

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27. 27% OptiPrep

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
46% OptiPrep	15006, Section 25	26.4 mL
DPBS/0.8M Salt Buffer (DPBS_0.8M)	15006, Section 24	18.6 mL

- 27.1. Combine 26.4 mL of 46% OptiPrep with 18.6 mL of DPBS/0.8M SALT BUFFER (DPBS_0.8M).
- 27.2. Protect from light. Select "Protected from light" on 15006-01.
- 27.3. Reagent expires 2 months from date of preparation and must be stored at room temperature.

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28. 33% OptiPrep

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
46% OptiPrep	15006, Section 25	32.2 mL
DPBS/0.8M Salt Buffer (DPBS_0.8M)	15006, Section 24	12.8 mL

- 28.1. Combine 32.2 mL of 46% OptiPrep with 12.8 mL of DPBS_0.8M.
- 28.2. Protect from light. Select "Protected from light" on 15006-01.
- 28.3. Reagent expires 2 months from date of preparation and must be stored at room temperature.

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29. 39% OptiPrep

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
46% OptiPrep	15006, Section 25	38 mL
DPBS/0.8M Salt Buffer (DPBS_0.8M)	15006, Section 24	6.8 mL

- 29.1. Combine 38 mL of 46% OptiPrep with 6.8 mL of DPBS_0.8M.
- 29.2. Protect from light. Select "Protected from light" on 15006-01.
- 29.3. Reagent expires 2 months from date of preparation and must be stored at room temperature.

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30. 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-PF)	Invitrogen, Cat # 21063-029	172 mL	430 mL
Heat Inactivated Fetal Bovine Serum (FBS)	Hyclone, Cat # SH30070.03HI	20 mL	50 mL
Glutamax I (Glut)	Invitrogen, Cat# 35-050-061	2 mL	5 mL
Antibiotic-Antimycotic (A/A)	Invitrogen, Cat # 15240-062	2 mL	5 mL
HEPES	Invitrogen, Cat # 15630-080	2 mL	5 mL
MEM, Non-Essential Amino Acids (NEAA)	Invitrogen, Cat # 11-140-050	2 mL	5 mL
0.2 µm PES Filter	VWR, Cat # 73520-988	1 unit	

- 30.1. Combine Phenol Red-Free DMEM-PF, FBS, Glutamax, Anti-Anti, HEPES, and MEM NEAA together into the top of a filter unit.
- 30.2. Filter media using a 0.2 µm PES Filter.
- 30.3. If preparing 500 mL, store in two separate aliquots to prevent accidental contamination if 500 mL is not used in a single batch run.
- 30.4. 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 ("30000: HPV Neutralization Assay for Titer Determination") and not shared between experiments or cell maintenance.

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31. 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, 26016	QS to 50 mL
0.22 µm Syringe Filter	Thomas Scientific, Cat # 1211K48	1 Unit
60 mL Syringe	Warehouse, Cat # 66301460	1 Syringe
60 mL PETG Bottle (60mL PETG)	Thomas Scientific, Cat # 1720N26	1 Bottle
Sodium Hydroxide (NaOH)	Sigma, Cat # 795429	As Needed

- 31.1. Dissolve Ammonium Sulfate into 45 mL of Type I Water in a 100 mL bottle.
- 31.2. Adjust pH to 9.0 ± 0.1 using Sodium Hydroxide dropwise, then QS to 50 mL with Type I Water.
- 31.3. Filter solution using a 0.22 µm Syringe Filter and 60 mL Syringe into a 60 mL PETG Storage Bottle.
- 31.4. Cover bottle with aluminum foil and select "Protected from light" on 15006-01.
- 31.5. Reagent expires 2 months from date of preparation and must be stored at 2-8°C.

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32. DPBS+1%BSA (DILUENT)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
1X Dulbecco's Phosphate-Buffered Saline (DPBS)	Gibco Cat # 14190-136	500 mL
Bovine Serum Albumin (BSA)	GeminiBio, Cat # 700-100P	5 ± 0.15 g
0.2 µm PES Filter	Thomas Scientific, Cat # 1234K60	1 unit

- 32.1. Open 1X DPBS bottle and pour powdered BSA into the bottle, mix to dissolve.
- 32.2. Filter using a 0.2 µm PES Filter.
- 32.3. Reagent expires 3 months from date of preparation and must be stored at 2-8°C.

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33. 10% TWEEN® 20 (10_T20)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
1X Dulbecco's Phosphate-Buffered Saline (DPBS)	Gibco, Cat # 14190-136	90 mL
TWEEN® 20 (T20)	VWR, Cat # EM-9480	10 mL

- 33.1. Add 90 mL of 1X DPBS to a container.
- 33.2. Add 10 mL of TWEEN® 20 to container using a serological pipette. Rinse pipette well in the solution.
- 33.3. Protect from light (may use amber bottle or aluminum foil). Select "Protected from light" on 15006-01.
- 33.4. Reagent expires 6 months from date of preparation and must be stored at 2-8°C.

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34. PBS+0.05% TWEEN® 20 (PBS_0.05T)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Phosphate Buffered Saline pH 7.4, containing TWEEN® 20, dry powder (PBS_T)	Thomas Scientific Cat # C987D15	1 packet
Type I Water	Water System, 26016 or equivalent	1 L

- 34.1. Add 1 packet of Phosphate Buffered Saline pH 7.4, containing TWEEN® 20, dry powder to a container.
- 34.2. Add 1 L of Type I Water to container using a volumetric Flask. Gently swirl to mix to avoid creating bubbles or use overhead stirrer to mix contents.
- 34.3. Reagent expires 3 months from date of preparation and must be stored at room temperature.

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35. PEI with 5% Glucose (PEI)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Polyethylenimine, Linear, MW 25,000 (Poly)	Polysciences, Cat # 23966-1	0.3 ± 0.02 g
Distilled Water (DH ₂ O)	Gibco, Cat # 15-230-001	300 mL
Hydrochloric Acid (HCl)	Sigma, Cat # H9892	As Needed
Sodium Hydroxide (NaOH)	Sigma, Cat # 795429	As Needed
D-(+)-Glucose (Glucose)	Sigma, Cat # G7021-1KG	15 ± 0.5 g
0.2 µm PES Filter	Thomas Scientific, Cat # 1234K58	1 unit

- 35.1. Dissolve Poly in Distilled water in a glass bottle. Drop pH to 2.4 ± 0.2 with HCl.
- 35.2. Warm mixture on stir plate. Utilize stir bar to maintain mixing for approximately 2 hours.
- 35.3. Once Poly has dissolved, allow mixture to reach room temperature then pH solution using NaOH to 7.2 ± 0.1.
- 35.4. Add Glucose to solution and mix until dissolved.
- 35.5. Filter using a 0.2 µm PES Filter.
- 35.6. Prepare 10 mL aliquots.
- 35.7. Reagent expires 1 year from date of preparation and must be stored at -10 to -30°C.

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36. 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

- 36.1. Combine 1X DPBS and 5M NaCl together into the top of a filter unit.
- 36.2. Filter using a 0.2 µm PES Filter.
- 36.3. Reagent expires 2 months from date of preparation and must be stored at 2-8°C.

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37. 50 MG SULFO-NHS

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Sulfo-NHS (N-hydroxysulfosuccinimide)	ThermoFisher, Cat # 24510	500 mg
Distilled Water (DH ₂ O)	Gibco, Cat # 15-230-001	10 mL

- 37.1. Add 10 mL of Distilled Water to Sulfo-NHS bottle.
- 37.2. Vortex until dissolved.
- 37.3. Make 600 µL aliquots into screw-top tubes for one-time use.
- 37.4. Reagent expires 1 year from date of preparation and must be stored at 2-8°C.

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38. DPBS+1% Triton X-100 (DPBS_1%TX)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
1X Dulbecco's Phosphate-Buffered Saline (DPBS)	Gibco, Cat # 14190-136	49.5 mL
Triton X-100	Sigma/ Thomas Scientific, Cat # C987P43	500 µL

38.1. Add 500 µL Triton X-100 to 49.5 mL of DPBS.

38.2. Reagent expires 9 months from date of preparation and must be stored at 2-8°C.

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39. 50 mM MES

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
MES-Hydrate	Sigma / Thomas Scientific, Cat # C988Z04	1.22 ± 0.01 g
Distilled Water (DH ₂ O)	Gibco, Cat # 15-230-001	125 mL
Hydrochloric Acid (HCl)	Sigma, Cat # H9892	As Needed
Sodium Hydroxide (NaOH)	Sigma, Cat # 795429	As Needed

- 39.1. Add 1.22 ± 0.01 g of MES-Hydrate to 125 mL of distilled water in an appropriately sized bottle.
- 39.2. Vortex until dissolved.
- 39.3. Adjust pH with HCl or NaOH to 5.0 ± 0.1.
- 39.4. Reagent expires 6 months from date of preparation and must be stored at 2-8°C.

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40. Histidine Storage Buffer

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
L-Histidine	Sigma, Cat # H8000-25G	248 ± 2.5 mg
Bovine Serum Albumin (BSA)	GeminiBio, Cat # 700-100P	800 ± 8.0 mg
5M Sodium Chloride (NaCl)	KD Medical, Cat # RGF-3270	8 mL
Distilled Water (DH ₂ O)	Gibco, Cat # 15-230-001	72 mL
Hydrochloric Acid (HCl)	Sigma, Cat # H9892	As Needed
Sodium Hydroxide (NaOH)	Sigma, Cat # 795429	As Needed
0.2 µm PES Filter	VWR, Cat # 73520-988	1 unit

- 40.1. Weigh out 248 ± 2.5 mg of L-Histidine and add it to a container.
- 40.2. Weigh out 800 ± 8.0 mg of BSA and add it to the container.
- 40.3. Add 8 mL of 5 M NaCl to the container.
- 40.4. Add 72 mL of distilled water to the container.
- 40.5. Mix until dissolved.
- 40.6. Adjust pH with HCl or NaOH to 6.2 ± 0.1.
- 40.7. Filter sterilize.
- 40.8. Reagent expires 1 year from date of preparation and must be stored at 2-8°C.

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41. Sheath Fluid

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Sheath Concentrate (20x Sheath)	Luminex, Cat # 40-50018	1 L
Preservative Concentrate (Pres)	Luminex, Cat # 40-50018	8 mL
Type II Water	Water System, 26016 or equivalent	QS 20 L

- 41.1. Add 1 L of Sheath Concentrate to the container.
- 41.2. Add 8 mL of Preservative Concentrate to the container.
- 41.3. QS to 20 L with Type II Water.
- 41.4. Reagent expires 3 months from date of preparation and must be stored at room temperature.

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42. PBST-BSA Buffer (PBST_BSA_PAK) Using Dry Powder Packets

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Bovine Serum Albumin (BSA)	GeminiBio, Cat # 700-100P	10 g
Phosphate Buffered Saline pH 7.4, containing TWEEN® 20, dry powder (PBS_T)	Thomas Scientific Cat # C987D15	1 packet
Type II Water	Water System, 26016 or equivalent	1 L

- 42.1. Add 10 g of BSA to an appropriately sized container.
- 42.2. Add 1 packet of Phosphate Buffered Saline pH 7.4, containing TWEEN® 20, dry powder to the container.
- 42.3. Add 1 L of Type II Water to the container using a volumetric Flask. Gently swirl to mix to avoid creating bubbles.
- 42.4. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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43. PBST-BSA Buffer (PBST_BSA)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Bovine Serum Albumin (BSA)	GeminiBio, Cat # 700-100P	10 ± 0.1 g
1X Dulbecco's Phosphate-Buffered Saline (DPBS)	Gibco Cat # 14190-136	1 L
TWEEN® 20 (T20)	VWR, Cat # EM-9480	500 µL
0.2 µm PES Filter	Thomas Scientific Cat # 1234K59	1 unit

- 43.1. Add 10 ± 0.1 g of BSA and 500 µL of TWEEN® 20 to 1 L of DPBS.
- 43.2. Mix the solution well by inversion to homogenize. Once the BSA has completely dissolved, filter sterilize the solution using a 0.2 µm PES Filter unit.
- 43.3. Reagent expires 1 month from date of preparation and must be stored at 2-8°C.

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44. PBS+0.05% TWEEN® 20 (Luminex_Wash)

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Phosphate Buffered Saline pH 7.4, containing TWEEN® 20, dry powder (PBS_T)	Thomas Scientific Cat # C987D15	1 packet
Type II Water	Water System, 26016 or equivalent	1 L

- 44.1. Add 1 packet of Phosphate Buffered Saline pH 7.4, containing TWEEN® 20, dry powder to a container.
- 44.2. Add 1 L of Type II Water to container using a volumetric Flask. Gently swirl to mix to avoid creating bubbles.
- 44.3. Reagent expires 3 months from date of preparation and must be stored at room temperature.

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45. HPV Plate Coating

Chemical/Consumable	Source / Cat # (or equivalent)	Amount
Coating Buffer	15006, Section 12	As calculated
HPV-Type Specific Virus-Like Particle (VLP)	Process Specific Procedure	As calculated
96-Well Plate	Thomas Scientific, Cat # 6925A00	As needed
Plate Sealer	Thomas Scientific, Cat # 6980A01	As needed

45.1. Refer to “30001: HPV Antibody ELISA” to determine VLP final coating concentration. Use form “15006.02: Plate Coating Form” to record plate coating information.

45.2. Calculate total volume needed using the following formula: # of plates x 96 wells/plate x 100 µL/well ÷ 1000 µL/mL + Overage. Overage can be scaled as needed. Round volume (mL) to the nearest whole number.

For example, to coat 6 plates: 6 plates x 96 wells/plate x 100 µL/well ÷ 1000 µL/mL + 4 mL overage = 61.6 mL, round up to 62 mL.

45.3. Calculate the volume of VLP needed using the following formula: $V_1 = C_2V_2 / C_1$, where C_1 is the starting VLP concentration, C_2 is the final VLP coating concentration, and V_2 is the total volume calculated in step 44.2. Multiply the value by 1000 µg/mL. Round volume (µL) to the nearest whole number.

For example, to coat 6 plates at a final VLP concentration of 2.7 µg/mL, with a starting VLP concentration of 3850 µg/mL:

$$V_1 = ((2.7 \mu\text{g/mL} \times 62 \text{ mL}) / 3850 \mu\text{g/mL}) \times 1000 \mu\text{g/mL} = 43 \mu\text{L of VLP needed}$$

45.4. In a BSC, add the total calculated volume of coating buffer to a container. Remove the calculated volume of VLP needed from the coating buffer.

45.5. Add the calculated volume of VLP to the coating buffer. Mix by inversion.

45.6. Using a multichannel pipette, add 100 µL of coating solution to each well of the 96-well plate. Cover plate with a plate sealer.

45.7. Label plates with HPV-type, Reagent Lot Number, Plate Use Dates, and Analyst Initials/date. Visually inspect each plate for coverage.

Note: “Plate Use Dates” refers to the date range that a plate can be used.

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Note: Plates are stored at 2-8°C and must be used between Day 3 and 5 from the coating date.

46. Blocking Buffer, 4% Skim Milk with 0.2% TWEEN® 20 in DPBS

Chemical/Consumable	Source / Cat # (or equivalent)	6 Plates	Standard Prep
Skim Milk Powder (Skim Milk)	BD, Cat # 232100	16 ± 0.4 g	1.0 ± 0.4 g
1X Dulbecco's Phosphate-Buffered Saline (DPBS)	Gibco Cat # 14190-136	400 mL	25 mL
TWEEN® 20 (T20)	VWR, Cat # EM-9480	800 µL	50 µL

- 46.1. For 6 plates, weigh out 16 ± 0.4 g of Skim Milk and add it to an appropriately sized container.
- 46.2. Add 400 mL of 1X DPBS to the container.
- 46.3. Mix vigorously until Skim Milk is fully dissolved.
- 46.4. Once solution is homogenous, add 800 µL of TWEEN® 20. Invert slowly to avoid producing excessive bubbles in the solution.
- 46.5. Allow Blocking Buffer to sit at room temperature for at least 30 minutes before use.
- 46.6. Reagent expires 24 hours from date of preparation and must be stored at 2-8°C overnight. Select "2-8°C" on 15006-01.

Note: Can scale total volume up or down as needed.

Note: Volume of Blocking Buffer for 6 plates has enough overage to use for weekly standard preparation (30001-02).

47. ATTACHMENTS

- 47.1. Attachment 1: Reagent Label Example
- 47.2. Attachment 2: 15006-01: Reagent Preparation Form
- 47.3. Attachment 3: 15006-02: HPV Plate Coating Form

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48. REVISION HISTORY

Version	Change	Reason
1.0	<ol style="list-style-type: none"> 1. Minor grammar and formatting changes throughout document. 2. Added EHS-WM-1 and EHS-WM-2 to references and Safety Considerations section. 3. Changed "HPV Serology Laboratory" to "FNL Serology Laboratory." 4. Edited 2N H₂SO₄ prep, Section 14, to empty the ampule into the 500 mL volumetric flask and then QS to 500mL with Type I water. 5. Added a PBNA_BSA_PAK prep section (new section 41) for prep without dry powder packets. 6. Changed PBS+0.05% TWEEN® preparation, section 33, to use Type I water. 7. Added section (new section 43) for prep of PBS+0.05% TWEEN® with Type II water. 8. Form 15006-01: added "Section" to the N/A of pH and Balance use, added note to printout attachment section for pH and balances without printers. 9. Form 15006-02: added additional equipment IDs, removed overage amount, added HSL_LAB_023 and changed Revision to Version. 10. Updated HPV Plate Coating (Section 44) with additional information. 11. Removed -80°C from Section 6. 12. Revised the expiration and storage temperature statement for each reagent. 13. Added Blocking Buffer Preparation (Section 45). 14. Formatted TWEEN® 20 the same throughout the document. 15. Updated SOP Document ID from HSL_GL_006 to 15006. 	<ol style="list-style-type: none"> 1. Clarification, ease of use. 2. Clarification for Fort Detrick laboratory. 3. Clarification for expansion of laboratory. 4. Clarification, ease of use. 5. Used in Neutralization Assay; to reflect current practice. 6. New Antibody ELISA assay developed. 7. Clarification for reagent used in Luminex Multiplex assay. 8. Clarification, ease of use. 9. Clarification, ease of use. 10. Clarification, ease of use. 11. No reagents currently stored at -80°C. 12. Consistency throughout SOP. 13. Ease of use. 14. Consistency throughout SOP. 15. Capture latest Document Naming convention.

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Attachment 1: Reagent Label Example

Reagent Preparation Label:

Reagent Name: _____
Lot Number: _____
Expiration Date: _____
Storage: _____
Analyst/Date: _____

Reagent Aliquot Label:

Reagent Name: _____
Lot #: _____
Expiration Date: _____
Analyst/ Aliquot Date: _____

Note: Due to label size, field descriptors such as “Reagent Name” and “Analyst/Aliquot Date” may not fit on the Aliquot Label; however, the reagent information must be recorded on the label regardless of field descriptors being present. See example below.

Example Aliquot Label:

70% Ethanol Lot #: 13Apr17-01 Exp: 13Apr18 ABC 13Apr17

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Attachment 2: 15006-01: Reagent Preparation Form

Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i>		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: Reagent Preparation Form			
Document ID: 15006-01		Version:	1.0
Associated SOP: 15006		Effective Date:	18Nov21
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Reagent Name:			
Preparation Date:		Expiration Date:	
Storage Condition:	<input type="checkbox"/> 2-8°C <input type="checkbox"/> RT <input type="checkbox"/> -10°C to -30°C <input type="checkbox"/> -65°C to -90°C <input type="checkbox"/> Protected from light		
Chemical / Consumable	Lot Number	Expiration Date	Amount Used
	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	
	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A

Comments:
 Reagent not included in 15006; see attached preparation protocol.
 N/A

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Effective Date: 18Nov21

Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i>		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: Reagent Preparation Form			
Document ID: 15006-01		Version:	1.0
Associated SOP: 15006		Effective Date:	18Nov21
Supersedes:	New	Page 2 of 3	

Balance: N/A Section

Equipment ID	Calibration Due Date
Affix Print out: <input type="checkbox"/> N/A, balance does not have printer	

pH Meter: N/A Section

Equipment ID	Required pH	Final pH
Affix Print out: <input type="checkbox"/> N/A, pH does not have printer		

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SOP Title: Reagent Preparation

Document ID: 15006

Version

1.0

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Supersedes

New

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Equipment N/A Section

Instrument Type	Equipment ID	Calibration Due Date
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A

Performed by/date:	
Reviewed by/date:	

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Attachment 3: 15006-02: HPV Plate Coating Form

Frederick National Laboratory for Cancer Research <i>sponsored by the National Cancer Institute</i>		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: HPV Plate Coating Form			
Document ID: 15006-02		Version:	1.0
Associated SOP: 15006		Effective Date:	18Nov21
Supersedes:	New	Page 1 of 1	

Equipment

Description	Equipment ID	Calibration Due Date
BSC	<input type="checkbox"/> HSL_007 <input type="checkbox"/> HSL_008 <input type="checkbox"/> HSL_009 <input type="checkbox"/> HSL_017 <input type="checkbox"/> HSL_074 <input type="checkbox"/> HSL_075 <input type="checkbox"/> HSL_076 <input type="checkbox"/> HSL_093 <input type="checkbox"/> HSL_094 <input type="checkbox"/> Other:	
2-8°C Refrigerator	<input type="checkbox"/> HSL_029 <input type="checkbox"/> HSL_084 <input type="checkbox"/> HSL_087 <input type="checkbox"/> HSL_089 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Pipette: μL	PIP_	
<input type="checkbox"/> N/A Pipette: μL	PIP_	
<input type="checkbox"/> N/A Pipette: μL	PIP_	

Reagents

Reagent	Lot Number	Expiration Date
VLP, HPV-Type:		<input type="checkbox"/> N/A
Coating Buffer		
96-well Plate		<input type="checkbox"/> N/A

Preparation

Starting VLP Concentration (μg/mL) (A)	Target VLP Coating Concentration (μg/mL) (B)	Associated Procedure (Document ID and Version)
		<input type="checkbox"/> 30001 <input type="checkbox"/> 30012 <input type="checkbox"/> 30002 <input type="checkbox"/> Other: Version:
Volume of Coating Buffer Needed		
Number of Plates	x Wells/plate	x Vol./well (μL)
	x	96
		+ 1000 μL/mL
		+ 1000 μL/mL
Volume of VLP Needed (μL): (B x C) + A x 1000 μL/mL		
Date Coated:		
Plate Use Dates: (Day 3-5)		
Performed by/date:		
Reviewed by/date:		

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