SOP Title: Plasmid DNA Transfection in HEK293TT for VLP Production and Purification		
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1. PURPOSE

1.1. The purpose of this procedure is to describe how to transfect Human Papillomavirus (HPV) plasmid DNA coding for empty capsid into HEK293TT (293TT) cells to produce virus-like particles (VLPs).

2. SCOPE

2.1. This procedure applies to the HPV Serology Laboratory.

2.2. This procedure includes the transfection of plasmid DNA into the 293TT cell line, VLP production, maturation, and purification of VLPs via a density-based gradient.

3. REFERENCES

3.1. 26000: Biosafety Cabinet (BSC) Use and Maintenance

3.2. 26001: Operation, Use and Maintenance of C02 Incubators

3.3. 26033: Use and Maintenance of the Thermo Scientific Sorvall XTR Centrifuge

3.4. 26004: Use and Maintenance of the Cellometer Auto 2000

3.5. 26005: Use and Maintenance of a 2-8°C Refrigerator

3.6. 26030: Use and Maintenance of -80°C Freezers

3.7. 26007: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath

3.8. 26009: Use and Maintenance of Pipettes

3.9. 26012: Use and Maintenance of an Analytical & Precision Balance

3.10. 26013: Use and Maintenance of -20°C Freezers

3.11. 26015: Use and Maintenance of an Inverted Microscope

3.12. 26016: Operation, Use and Maintenance of the Water Purification Systems

3.13. 26017: Use and Maintenance of the Eppendorf Centrifuge

3.14. 26018: Use and Maintenance of NanoDrop Spectrophotometers

3.15. 26021: Use and Maintenance of the Optima XPN Ultracentrifuge System

3.16. 15000: Waste Disposal at the Advanced Technology Research Facility

3.17. 15006: Reagent Preparation for the HPV Serology Laboratory

3.18. 20001: 293TT Cell Culturing and Maintenance

3.19. 30009: BCA Protein Assay

3.20. 30010: Acrylamide Protein Gel Analysis of HPV Virus-Like Particles

3.21. 30011: Protein Analysis of Virus-Like Particles (VLPs) Using the Agilent 2100 Bioanalyzer

3.22. 10010: Lot Number and Test Run Number Assignment

3.23. 10009: General Record Review

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as Analyst, is responsible for reviewing and following this procedure and documenting assay information.

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.

4.4. Trained personnel perform assay record review per “10009: General Record Review.”

5. DEFINITIONS

Term	Definition
293TT	HEK 293TT cells
A/A	Antibiotic/Antimycotic
DMEM	Dulbecco’s Modified Eagle’s Medium with High Glucose
DMEM SF	Dulbecco’s Modified Eagle’s Medium with High Glucose Serum Free Media
DMEM TF	Dulbecco’s Modified Eagle’s Medium with High Glucose Transfection Media
PsV	Pseudovirions
RT	Room Temperature
Type II Water	Pure/Analytical Grade, used for standard applications (Resistivity >1 MΩ-cm and TOC ≤ 50 ppb)
VLP	Virus Like Particle

6. REAGENTS, MATERIALS AND EQUIPMENT

6.1. Reagents

6.1.1. 1M Ammonium Sulfate (15006: Section 30)

6.1.2. Benzonase (Sigma, Cat # E1014-25KU)

6.1.3. Bleach, Concentrated (FNLCR Warehouse, Cat # 68100251 or equivalent)

6.1.4. 10% Brij58 (15006: Section 23)

6.1.5. Dulbecco's Modified Eagle's Medium (DMEM) with: High Glucose (Gibco, Cat # 11965-112 or equivalent)

6.1.6. 1X Dulbecco's Phosphate-Buffered Saline (DPBS), Sterile (Life Technologies, Cat # 14190-136 or equivalent)

6.1.7. DPBS_0.8M (15006: Section 24)

6.1.8. DPBS-$MgCl_2$ 10mM A/A (DPBS_MgCl_AA) (15006: Section 22)

6.1.9. Expression Plasmid Coding HPV Capsid Sequences

6.1.10. Fetal Bovine Serum (FBS), Heat-Inactivated (Hyclone, Cat # SH30070.03HI or equivalent)

6.1.11. Glutamax I (Gibco, Cat # 35050-061 or equivalent)

6.1.12. HEK 293TT Cells (20001)

6.1.13. Lipofectamine 2000 (Life Technologies, Cat# 11668-019)

6.1.14. MEM Non-Essential Amino Acids (NEAA) (Gibco, Cat # 11140-050 or equivalent)

6.1.15. 5M NaCl (KD Medical, Cat # RGF-3270 or equivalent)

6.1.16. Opti-MEM (Life Technologies, Cat # 11058-021)

6.1.17. 27% OptiPrep (15006: Section 26)

6.1.18. 33% OptiPrep (15006: Section 27)

6.1.19. 39% OptiPrep (15006: Section 28)

6.1.20. Plasmid-Safe DNase (Epicentre Biotechnologies, Cat # E3101K)

6.1.21. Polyethylenimine with 5% Glucose (PEI) (15006: Section 34)

6.1.22. Primary Disinfectant (Cavicide, FNLCR Warehouse, Cat # 79300360 or equivalent)

6.1.23. Secondary Disinfectant (Ster-ahol, VWR, Cat # 14003-358 or equivalent)

6.1.24. Transporter 5 (Polysciences, Inc., Cat # 26008-50)

6.1.25. Trypsin-EDTA 0.05% (Life Technologies, Cat # 25300-054 or equivalent)

6.1.26. Type II Water

6.2. Equipment

6.2.1. Automated Cell Counter (Nexcelom Cellometer Auto 2000 or equivalent)

6.2.2. Cannulas (VWR, Cat # 20068-680 or equivalent)

6.2.3. Centrifuges (Microcentrifuge, Bench Top, Ultracentrifuge)

6.2.4. Class II Biosafety Cabinet (BSC)

6.2.5. CO_2 Incubator

6.2.6. Filtering Flask with plug (Thomas Scientific Cat # 4949J26 or equivalent)

6.2.7. Freezers (-20°C, -80°C)

6.2.8. Ice Pan (Thomas Scientific, Cat # 1200R42 or equivalent)

6.2.9. Inverted Light Microscope (Nikkon TMS, Nikkon LBOPHOT or equivalent)

6.2.10. -20°C Labtop Cooler (Thomas Scientific Cat # 5152L78 or equivalent)

6.2.11. NanoDrop

6.2.12. Pipettes

6.2.13. Precision Balance

6.2.14. Refrigerator (2-8°C)

6.2.15. Rotor SW40 Ti, rated for > 200,000 x g

6.2.16. Rotor SW55 Ti, rated for > 200,000 x g

6.2.17. Serologic Pipettor

6.2.18. Stand with clamp

6.2.19. Timer

6.2.20. Waste Container with Lid

6.2.21. Water Bath

6.2.22. Water Purification System

6.2.23. Wide screwdriver, Hinge Pin Tool (Beckman Cat # 331313 or equivalent)

6.2.24. Puncture Resistant Glove

6.3. Consumables

6.3.1. S1 Pipet Filler (Thermo Scientific, Cat # 9511 or equivalent)

6.3.2. 150 mL, 250 mL, 500mL and 1L Bottles, Media Storage (Thomas Scientific, Cat # 8600B13, Cat # 1743D05, Cat # 1743D10, Cat # 1743D15 or equivalent)

6.3.3. 2 L Bottle, Media Storage - Corning Polystyrene Roller Bottle (VWR, Cat # 89184-640 or equivalent)

6.3.4. Box and 81 Positions Insert (FNLCR Warehouse, Cat # 81150001 and 81150004 or equivalent)

6.3.5. 250 mL, 500 mL, and 1L Filter Bottle, 0.2 µM PES Membrane - Nalgene (Thomas Scientific, Cat # 1234K60, Cat # 1234K58, Cat # 1234K59 or equivalent)

6.3.6. T-150 Flask, T-225 Flasks (Thomas Scientific, Cat # 9381J33, Cat # 9381M60 or equivalent)

6.3.7. 5-Layer Flask (VWR, Cat # 89204-478 or equivalent)

6.3.8. 8-Layer Flask, CELLdisk (Greiner Bio-One, Cat # 678108 or equivalent)

6.3.9. Kimwipes, Lint-Free Lens Wipe (VWR, Cat # 21905-026 or equivalent)

6.3.10. Parafilm (FNLCR Warehouse, Cat # 66401356 or equivalent)

6.3.11. Pipette Tips

6.3.12. Disposable Serum Pipette (Greiner Bio-one, Cat # 612361 or equivalent)

6.3.13. 5 mL, 10 mL, 25 mL, and 50 mL Serological Pipets, Sterile (FNLCR Warehouse, Cat # 66401365, Cat # 66401370, Cat # 66401361, Cat # 66401363 or equivalent)

6.3.14. 100 mL Serological Pipets, Sterile (Thomas Scientific, Cat # 7536R26 or equivalent)

6.3.15. Siliconized 200 µL and 1000 µL Pipette Tips (Thomas Scientific, Cat # 7738E15, Cat # 7738E30 or equivalent)

6.3.16. Siliconized 1.5 mL and 2.0 mLTubes, Flip-Cap (Thomas Scientific, Cat # 1212M66, Cat # 1212M73, or equivalent)

6.3.17. 1 mL Syringe with 25-Gauge Needle and 10 mL Syrninge - BD (FNLCR Warehouse, Cat # 66301465, Cat # 66301450, or equivalent)

6.3.18. Sharps container (FNLCR Warehouse, Cat # 66401504 or equivalent)

6.3.19. 50 mL Tubes, Conical Centrifuge (FNLCR Warehouse, Cat # 66401493 or equivalent)

6.3.20. 50 mL and 500 mL Tubes, Conical Centrifuge, Polystyrene (Thomas Scientific, Cat # 1216M90, Cat # 8600A70, or equivalent)

6.3.21. 5 mL Tubes, and 14 mL Thinwall Polypropylene (Ultratubes) (Beckman Coulter, Cat # 326819 and Cat # 331374)

6.3.22. Vacushield Vent with PTFE (PALL, Cat # 4402 or equivalent)

6.3.23. Wet Ice

6.3.24. Wipe, Low-Lint, Wypalls (FNLCR Warehouse, Cat # 79300335 or equivalent)

7. HEALTH AND SAFETY CONSIDERAIONS

7.1. 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, safety glasses, closed-toe shoes, and non-latex gloves.

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).

8. PROCEDURE PRINCIPLES

8.1. Transfection can be performed using one of three reagents. Refer to the respective attachment throughout the procedure.

8.1.1. See Attachment 1 for Lipofectamine 2000 process-related information.

8.1.2. See Attachment 2 for PEI process-related information.

8.1.3. See Attachment 3 for Transporter 5 process-related information.

8.2. Lot numbers are assigned per “10010: Lot Number and Test Run Number Assignment.”

8.3. All contaminated BSL-2 level liquid waste must be decontaminated using 10% Clorox bleach (final concentration) with a minimum contact time of 30 minutes in waste container before sink disposal. Refer to “15000: Waste Disposal at the Advanced Technology Research Facility.”

8.4. Acceleration and Deceleration using centrifuges

8.4.1. When centrifuging conical tubes using the Sorvall XTR centrifuge per "26033: Use and Maintenance of the Thermo Scientific Sorvall XTR Centrifuge", use maximum ramp up/acceleration speed and break speed of 6 or higher.

8.4.2. When centrifuging 1.5 mL tubes using the microcentrifuge per "26017: Use and Maintenance of the Eppendorf Centrifuge", use maximum ramp up/acceleration speed and maximum break speed.

8.5. When working with 5 or more flasks, process in batches.

9. REAGENT PREPARATION

9.1. Warm all tissue culture medium to room temperature prior to use if stored 2-8°C.

9.2. DMEM TF, DMEM 2%, and DMEM SF Medium Preparation

9.2.1. Combine the following reagents according to Table 1 for **500 mL** volume. Scale volumes as needed.

Table 1: Media Preparation

	DMEM TF	**DMEM 2%**	**DMEM SF**
Reagent Name	**Volume 500 (mL)**	**Volume 500 (mL)**	**Volume 500 (mL)**
DMEM	440	480	490
FBS	50	10	0
NEAA	5	5	5
Glutamax	5	5	5
0.2 µm PES Filter unit	1 unit	1 unit	1 unit

9.2.2. Filter with 0.2 µm PES filter.

Note: If making larger volumes, the same 0.2 µm PES filter can be used.

9.2.3. Label with reagent name, data reference, date/time, and Analyst initials.

9.2.4. Prepare reagent immediately prior to use and maintain at 2-8°C if not used same day. Use within 24 hours.

9.3. Transfection Lysis Buffer (Cell Harvest, Day 4, Section 12)

9.3.1. Combine the following reagents according to Table 2 (scale as needed).

Note: The 10% Brij58 needs to be prepared 24 hours in advance.

Table 2: Transfection Lysis Buffer Preparation

Lysis Buffer Total Volume	**1 mL**
DPBS_MgCl_AA	0.884 mL
10% Brij58	0.067 mL
Benzonase	4.2 µL
DNase	4.2 µL
1M Ammonium Sulfate (NH_3SO_4)	41.7 µL

9.3.2. Label with reagent name, data reference, current date, and Analyst initials.

9.3.3. Prepare reagent immediately prior to use and maintain on wet ice or 2-8°C. Discard remaining Lysis Buffer after use.

Note: If making larger volumes, the same 0.2 µm PES filter can be used.

10. 293TT CELL PREPARATION (DAY 1)

Note: Enter pertinent information on "2005: HEK293TT Transfection Form, Day 1-4."

10.1. Refer to "20001: 293TT Cell Culturing and Maintenance" for information regarding the harvesting, counting, and seeding of HEK 293TT cells.

10.2. Seed cell culture flasks per the respective attachment (Section 8.1) in DMEM TF per Table 3.

Table 3: Volume of DMEM TF to add to each flask

Flask Type	**DMEM TF (per flask)**
T-150	20-30 mL
T-175	25-35 mL
T-182	25-35 mL
T-225	30-40 mL
5-layer	120-140 mL
8-layer	270-300 mL

10.3. Incubate cells overnight (16-24 hours) in a 37°C, 5% CO_2 incubator.

11. TRANSFECTION (DAY 2)

11.1. Refer to the respective attachment (Section 8.1) to confirm 293TT confluency via inverted microscope per "26015: Use and Maintenance of an Inverted Microscope." If confluency is below confluency range, allow cells to grow until appropriate confluency is reached.

Note: Report confluency in multiples of 5 (85%, 90%, etc.).

11.2. Thaw HPV plasmid on wet ice then mix by inversion.

11.3. Confirm concentration of DNA using NanoDrop per "26018: Use and Maintenance of NanoDrop Spectrophotometers."

11.4. Affix NanoDrop data to 20005-01.

11.5. Prepare Transfection Cocktail.

11.5.1. Prepare Transfection Reagent:Media.

Note: "Transfection Reagent" can refer to either Lipofectamine 2000, PEI, or Transporter 5.

11.5.1.1. Prepare Transfection Reagent:Media mixture as shown in the respective attachment (Section 8.1). Invert to mix.

11.5.1.2. Incubate Transfection Reagent:Media mixture for 5-10 minutes at room temperature.

Note: Do not allow Transfection Reagent from step 11.5.1.2 to sit in Media longer than 25 minutes.

11.5.2. Prepare DNA:Media mixture as shown in the respective attachment (Section 8.1). Invert to mix.

11.5.3. Add Transfection Reagent:Media mixture to DNA:Media mixture into an appropriate-sized flask or bottle (this is now called **Transfection Cocktail**). Invert to mix.

11.5.4. Incubate Transfection Cocktail at room temperature for 20-30 minutes.

11.6. Remove 293TT flasks from incubator and place in BSC.

11.7. Add Transfection Cocktail.

11.7.1. Gently swirl to mix Transfection Cocktail. Handle conditioned media as per 11.7.1.1 – 11.7.1.2. Add Transfection Cocktail to 293TT flasks according to Transfection reagent used, see attachment (Section 8.1).

11.7.1.1. When using Lipofectamine 2000, leave conditioned media on cells during Transfection Cocktail incubation.

11.7.1.1.1. For T225 flask, add Transfection Cocktail directly to the side of the flask do not disturb cells, and then gently rock flask to mix.

11.7.1.1.2. For multi-layer flasks, transfer culture media to a sterile storage media bottle and add Transfection Cocktail into culture media. Invert to mix, and slowly transfer back to culture flask.

11.7.1.2. When using PEI or Transporter 5, remove conditioned media prior to adding Transfection Cocktail.

11.7.1.2.1. Remove conditioned media from flask using a serological pipet or by decanting into waste container.

11.7.1.2.2. Add Transfection Cocktail to each flask, careful not to disturb cells, and gently rock flask to cover cells completely.

11.8. Label each flask with cell type, transfection date, transfection reagent, plasmid name, Data Reference/Lot Number, and Analyst initials per Attachment 4.

11.9. Incubate cells in a 37°C, 5% CO_2 incubator for 5-6 hours.

Note: Use the start time of the first flask transfected on 20005-01.

Note: The start time of the Transfection at 37°C (Step 11.9) is also the start time of the 48 ± 2 hour Transfection Incubation at 37°C (Step 11.12).

11.10. Remove flasks from incubator and place in BSC.

11.11. Process flasks according to Transfection reagent used as per 11.11.1 – 11.11.2.

11.11.1. When using Lipofectamine 2000, remove media using a serological pipet, or by decanting, into waste container. Add room temperature DMEM TF to each flask per Table 4.

11.11.2. When using PEI or Transporter 5, leave transfection cocktail on the cells. Add sufficient quantity of room temperature DMEM TF to flask to achieve the final volume as indicated in Table 4.

Table 4: Final Volume of DMEM TF

Flask Type	DMEM TF (per flask)
T150	28 mL
T175	32 mL
T182	34 mL
T225	40 mL
5-Layer	180 mL
8-Layer CELLdisk	405 mL

11.12. Incubate transfected cells in 37°C, 5% CO_2 incubator for 48 ± 2 hours using the incubation start time from Step 11.9.

12. CELL HARVEST (DAY 4)

12.1. Place flasks in BSC. Remove media using a serological pipet or by decanting into an appropriately-sized conical centrifuge tube, labeled "**A**"

12.2. Gently wash attached cells with DPBS.

12.2.1. Add volume of DPBS per Table 5 to each flask.

Table 5: Volume of DPBS to add to each flask

Flask Type	Volume of DPBS
T150-T225	5-15 mL
5-Layer	20-40 mL
8-Layer CELLdisk	40-60 mL

12.2.2. Gently wash attached cells by rocking back and forth 3-5 times.

12.2.3. Collect wash using serological pipet or decanting the supernatant into a conical centrifuge tube, labeled "**B**".

12.2.4. Repeat DPBS wash, steps 12.2.1 and 12.2.2 if needed.

12.3. Add trypsin to flask per Table 6. Gently rock flasks to distribute trypsin evenly over cells and incubate for 3-5 minutes in the 37°C, 5% CO_2 incubator. Prolonged exposure to trypsin approaching 25 minutes is toxic to cells.

Table 6: Volume of Trypsin to add to each flask

Flask Type	Volume of Trypsin
T150-T225	3-5 mL
5-Layer	12-20 mL
8-Layer CELLdisk	27-45 mL

12.4. Place flask into BSC. Wash flasks with media from container “A” to collect detached cells and add into container “B.” Use volumes listed in Table 5.

12.5. Visually confirm that cells have detached from the flask per 26015.

12.5.1. Repeat step 12.4 as needed to collect cells that are still attached.

12.6. Repeat steps 12.1 to 12.5 for remaining flasks. Pool cells into same container “B”; use additional centrifuge tubes as needed.

12.7. Centrifuge balanced tubes at 300 x g for 10 minutes at 20°C per 26033.

12.8. Slowly decant media from centrifuge tubes into waste container. If pellet dislodges from bottom of conical tube, use serological pipet to remove media to prevent pellet loss.

12.9. Add 5 mL of DPBS if using a 50 mL conical tube, or 15 mL of DPBS if using a 500 mL bottle, to cell pellets. Gently resuspend cells via swirling the tube, finger tapping, or using a serological pipet. Cells may be consolidated into a 50mL conical tube.

12.10. Centrifuge tubes at 300 x g for 10 minutes at 20°C per 26033.

12.11. Use serological pipet or slowly decant supernatant into waste container. If decanting, ensure residual fluid is removed via serological pipet.

Note: The pellet will not be strongly adherent to tube.

12.12. Estimate the volume of pellet by comparing to fluid of a known volume in a tube the same size. Add 1.5 times the cell-pellet volume with Transfection Lysis Buffer (See 9.1) (Example: Add 1.5 mL of Transfection Lysis Buffer to 1 mL of cell pellet).

12.13. Gently mix the cell pellet and lysis buffer mixture via serological pipet or pipette.

12.14. Label 1.5 mL siliconized tubes with Lysate Tube label (see Attachment 4 for example).

12.15. Aliquot 1 mL of lysate into each 1.5 mL siliconized tube using siliconized pipette tips, then wrap each tube lid with parafilm. Make note on tube if volume is other than 1 mL.

12.16. Incubate tubes for 22-26 hours in a 37 ± 2°C water bath per "26007: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath" for VLP maturation.

Note: Invert tubes 1-2 times within the first two hours of incubation to ensure uniform lysis and exposure to lysate reagents.

Note: Some HPV types may need a longer maturation; ask Scientific Manager. Document any procedural changes on 20005.01.

12.17. Remove parafilm and wipe each tube with Ster-ahol, then transfer tubes to wet ice or to a 2-8°C refrigerator per "26005: Use and Maintenance of a 2-8°C Refrigerator" for 10-20 minutes.

12.18. Add 0.175 mL of 5M NaCl to each 1 mL of lysate and gently mix by finger tapping tube or inverting 3-5 times. Do not pipet up and down to mix.

Note: Keep same ratio of NaCl if volume of lysate is not 1 mL. For example, if lysate volume is 0.8 mL use 0.14 mL of 5M NaCl.

12.19. Incubate tubes for 10-20 minutes on wet ice or in a 2-8°C refrigerator per 26005.

12.20. Proceed to VLP purification (Section 13 Day 5) for immediate processing or transfer tubes to labeled box (Attachment 4) and freeze tubes in -80°C freezer for subsequent purification.

13. GRADIENT AND PURIFICATION (DAY 5)

Note: Enter pertinent information on "20005-02: HEK293TT Transfection Form, Day 5."

13.1. Ultracentrifuge Preparation

13.1.1. Turn on ultracentrifuge and prepare it for use per "26021: Use and Maintenance of the Optima XPN Ultracentrifuge System."

13.1.1.1. Select program per Table 7. Confirm settings for rotor type, speed, tube size, and temperature per 26021.

Table 7: Ultracentrifuge Gradient Program Settings

Program Name	Rotor Type	Ultratube Part Number / Size	Volume of Each Gradient to Use (µL)	Rotor Speed	Temperature	Length of time (hour : min)
HPV_PsV	SW55 Ti	326819 / 5 mL	1200	303,800 x g	16°C	03:30
HPV_PsV_SW40	SW40 Ti	331374 / 14 mL	2200	284,600 x g	16°C	04:45

13.1.1.2. Check vacuum prior to run by pressing Open Vent (vacuum display should read below 5 microns (0.7 Pa) prior to sample processing). Once complete, release vacuum (> 1,000 microns).

13.2. Gradient Preparation

13.2.1. Label Thinwall Polypropylene Tubes (ultratubes) with ultratube number.

13.2.2. Create a 27%, 33%, and 39% Opti-Prep step gradient, using volumes appropriate to tube size and rotor, per Table 9.

13.2.2.1. Using a sterile syringe fitted with a cannula, add 27% Opti-Prep to the bottom of ultratube.

13.2.2.2. Using a clean syringe fitted with a clean cannula, underlay 33% Opti-Prep by slowly dispensing to create a gradient.

13.2.2.3. Using a clean syringe fitted with a clean cannula, underlay 39% Opti-Prep by slowly dispensing to create a gradient.

Note: When held at eye level in BSC, an interface between gradients should be visible if gradient was dispensed slowly.

Note: Use the syringe to rinse the cannulas with Type II Water to prevent the Opti-prep from clogging cannulas.

13.2.3. Allow gradient to diffuse 1-2 hours at room temperature with minimal light exposure.

13.2.4. Turn on microcentrifuge and bring to 4°C per “26017: Use and Maintenance of the Eppendorf Centrifuge.”

13.2.5. If lysates are frozen, while gradient is diffusing, remove lysates from step 12.20 from-80°C freezer and thaw on wet ice, then invert to mix.

Note: SW55 Ti rotor can process about 7 lysate tubes, and the SW40 Ti rotor can process about 26-36 lysate tubes in one run.

13.2.6. Clarify lysate by centrifuging at 10,000 x g at 4°C for 10 minutes in the microcentrifuge per 26017.

Note: Use siliconized pipette tips or serological pipets whenever possible when pipetting lysate.

13.2.7. Pool Supernatant into new siliconized 1.5 mL tubes and repeat centrifugation per step 13.2.6.

13.2.7.1. Repeat step 13.2.7 until supernatant is clear of cell debris, if necessary.

13.2.7.2. Save Cell Pellets for use in steps 13.2.9-13.2.12.

13.2.8. Place Supernatant tubes into BSC. Remove clarified supernatant and transfer to 1.5 mL siliconized tubes or 50 mL polystyrene tube labelled “Supernatant.” Store “Supernatant” tube on wet ice.

13.2.9. Add 400 µL of DPBS_0.8M to Cell Pellets from first centrifugation (see 13.2.7).

Note: Gently mix each cell pellet using a pipette or mix by inversion.

13.2.10. Centrifuge cell pellets at 10,000 x g at 4°C for 10 minutes in microcentrifuge per 26017.

Note: A significant amount of VLPs may still be found in cell pellet and washing cell pellet ensures that most VLPs have been collected.

13.2.11. Remove clarified supernatant from tube and transfer to 1.5 mL siliconized tubes or a 50 mL polystyrene tube labelled “Cell Wash.” Store “Cell Wash” supernatant tube on wet ice.

13.2.12. Store all supernatants on wet ice until gradient has fully diffused.

13.2.13. Document content in ultratubes per “20005-02: HEK293TT Transfection Form, Day 5.”

13.3. Ultracentrifugation

13.3.1. Inside BSC, carefully add supernatants to top of Opti-Prep gradient using a siliconized pipette tip or serological pipet.

Note: Pipet supernatant slowly to ensure gradient is not disturbed.

13.3.1.1. Hold ultratube at a slight angle, 20 – 30 degrees from straight up, then slowly pipet supernatants along the edge of ultratube closest to horizontal.

13.3.1.2. As the supernatant layer is established, gradually bring ultratube to an upright position.

13.3.2. Fill all ultratubes to approximately 4 mm from the top to prevent tube from collapsing during ultracentrifugation. Carefully transfer ultratubes into ultracentrifuge buckets.

13.3.3. Use the Precision Balance, per “26012: Use and Maintenance of an Analytical & Precision Balance” to balance buckets including lids.

Note: Pair Bucket 1 with 4, Bucket 2 with 5, and Bucket 3 with 6.

13.3.4. Using a pipette add DPBS_0.8M to balance paired buckets containing tubes until pairs are equal weight.

13.3.5. Firmly tighten the bucket lid closed to prevent leaks using screwdriver.

13.3.6. Load buckets onto rotor and gently verify that buckets are hooked to rotor and swing freely.

Note: Do not spill lysate into buckets by allowing tube to swing too far from vertical.

13.3.7. Load rotor into ultracentrifuge, and select appropriate program corresponding to Table 8. Start ultracentrifuge run.

Note: To avoid disturbing gradient, use minimal brake.

13.3.8. Once program is completed, carefully remove rotor from ultracentrifuge. Document final run details in 26021-01.

13.4. Gradient Collection

13.4.1. Label twenty 1.5 mL siliconized tubes for each gradient tube processed: fractions 1-10, plus 20 µL aliquots of fractions 1-10.

Note: See Attachment 4 for label guidance.

13.4.2. Place ultratubes into BSC. Collect fractions from the bottom of ultratubes.

13.4.2.1. Gently but firmly secure ultratube with clamp and stand.

13.4.2.2. Carefully pierce a hole in the bottom of the Thinwall Polypropylene Tube (ultratube) with a 25G needle, while minimizing disruption to ultratube contents.

Note: Wear protective, puncture-resistant gloves when working with needles, and dispose of needles in sharps box per 15000.

13.4.3. Collect fractions at the suggested volumes in Table 8 via gravity-based dripping. Place fractions into ten labeled tubes, fractions 1-10 in order, from step 13.4.1.

Table 8: Suggested Fraction Volume Collection

Rotor Type	Tube P/N / Size	Volume of Fraction 1 to Collect (µL)	Volume of Fractions 2-10 to Collect (µL)
SW55 Ti	326819 / 5 mL	400	200
SW40 Ti	331374 / 14 mL	1000-1300	300

13.4.4. Gently mix fractions by inversion (do not vortex) or by pipet and aliquot approximately 20 µL of each fraction into tubes labeled for 20 µL aliquots from step 13.4.1 for in-process testing per “30010: Acrylamide Protein Gel Analysis of Virus-like Particles (VLPs).”

13.4.5. Store VLPs at -80°C in a box labeled per Attachment 4. Store the 20 µL aliquots at -80°C in a separate box labeled per Attachment 4.

13.4.6. Repeat sections 8 to 13 until desired VLP yield is obtained then proceed to section 14. For HPV-16 VLP about 8 X T225 flasks and for HPV-18 VLP about 17 X T225 flasks generate about 1 mg of VLP.

14. FRACTION POOLING

14.1. Pool fractions selected by Scientific Manager based on in-process testing results from 30010 and “30011: Protein Analysis of Virus Like Particles (VLPs) Using the Agilent 2100 Bioanalyzer”.

14.1.1. Affix pooled fraction selection table to “20005-03: Fraction Pool Form;” confer with Scientific Manager.

14.2. Thaw fractions on wet ice or 2-8°C. Combine fractions using siliconized tips into a polystyrene conical tube or storage bottle. Gently mix by inversion.

14.3. Label each tube according to Attachment 5.

Note: Lot numbers are assigned based on 10010.

14.4. Perform BCA and Agilent protein procedure(s) the same day as pooling per "30009: BCA Protein Assay" and 30011. Annotate protein concentration results on form 20005-03 from the BCA assay for VLP L1 proteins and the Agilent for VLP L1L2 on form 20005-03.

Note: Consult Scientific Manager for aliquot concentration.

14.5. Additional aliquots are prepared and stored at -80ºC for confirmatory testing as suggested in Attachment 5. Annotate aliquot information and any future testing results per 20005-03.

14.6. Dilute the Electron Microscopy fraction to 0.5 mg/mL in DPBS.

14.7. Record number of aliquots into Freezer Inventory and on 20005-03.

15. ATTACHMENTS

15.1. Attachment 1: Lipofectamine 2000 Transfection

15.2. Attachment 2: PEI Transfection

15.3. Attachment 3: Transporter 5 Transfection

15.4. Attachment 4: Transfection Flask, Lysate Tube and Box, and Fraction Tube and Box Label Guidance

15.5. Attachment 5: Pooled Fraction Tube and Box Label Guidance and Suggested Aliquots for Testing

15.6. Attachment 6: 20005-01: HEK293TT Transfection Form, Day 1-4

15.7. Attachment 7: 20005-02: HEK293TT Transfection Form, Day 5

15.8. Attachment 8: 20005-03: Fraction Pool Form

16. REVISION HISTORY

Version	Change	Reason

4.0	1. Added HSL_QS_022 to References and Procedure Principles section. 2. Updated volumes of DMEM preparations in section 9. 3. Section 10 medium volumes updated. 4. Attachments 1-3, confluency and T225 volumes updated. 5. Form HSL_LAB_005.01: changed Opti-MEM to Transfection Media and updated cell count section. Minor revision in day 2 incubation section.	1. Clarification. 2. Reflect current practice. 3. Reflect current practice. 4. Reflect current practice. 5. Reflect current practice.
4.1	1. Correct Version number. 2. Add Agilent, HSL_LAB_011, procedure and name to Reference section and step 14.4. 3. Add notes to step 11.8 to use start time of first flask as start time on HSL_LAB_005.01, and that 37°C 5-6 hour incubation start time is transfection incubation start time (part of the 48 ± 2 hour incubation). 4. Minor space formatting all forms. 5. HSL_LAB_005.01: For Post-Transfection DMEM Preparation, changed table title to “Post-Cell Culture Transfection DMEM TF Preparation”	1. Incorrect version at release. 2. Referenced in body of procedure. 3. Clarification. 4. Ease of use. 5. Clarification.
5.0	1. Section 2.1 removed location 2. Section 2.2 Revised wording 3. Updated SOPs used with new numbers 4. Updated responsibilities 5. Updated definitions 6. Removed DMEM 10A from Reagents 7. Added reagents and equipment used 8. Section 7.2 spelled out SDS 9. Section 7.2 updated SOP number 10. 8.2.1 added lot number assignment 11. Added 8.3-8.5 12. Added 9.1 for media preparation 13. Added 9.2.1 note 14. Table 1 was simplified 15. 9.2 Added data reference 16. 9.2.2 Added data reference 17. 9.2.3 Clarified wording 18. 9.3 combined tables 2-4 19. Section 10 created a table 20. 11.1 included section reference number	1. Reflect current practices 2. Clarification 3. Reflect current practices 4. Reflect current practices 5. Clarification 6. Reflect current practices 7. Reflect current practices 8. Clarification 9. Reflect current practices 10. Clarification 11. Clarification 12. Clarification 13. Clarification 14. Ease of use 15. Clarification 16. Clarification 17. Clarification 18. Ease of use 19. Ease of use 20. Ease of use

	21. 11.4 remove “raw” 22. 11.5.1.1 included attachment reference 23. 11.5.1.1 added mix step 24. 11.5.1.2 created note to separate text 25. 11.5.2 added mix step 26. 11.7 Described Conditioned media use 27. 11.8 removed passage number 28. 11.9 clarify note 29. 11.10 Clarified use of different reagents 30. 11.12 Added Step Reference 31. 12.1 Added conical centrifuge tube 32. Table 5 added T150 flask 33. 12.2.3 simplified wording 34. 12.3 added wording 35. Table 6 added T150 flasks 36. Removed step 12.4 37. 12.6 clarified process 38. 12.7 added “balanced” 39. 12.9 added consolidation step 40. 12.11 removed absorbent towel 41. 12.12 reword 42. 12.13 specify mixing step 43. 12.15 mix step removed redundant with previous step instructions 44. 12.15 Added note of volume differences 45. 12.16 made note to separate text 46. 12.16 notify scientific manager added 47. 12.17 reword for procedure process 48. 12.18 Added mix clarification and note 49. 12.20 added proceed to section 5 50. Added tube size 51. 13.1.1.2 Added vacuum parameters 52. 12.2.1 Label Added 53. 13.2.4 moved cool centrifuge sooner 54. 13.2.5 Added if lysates are frozen 55. 13.2.5 and 13.2.6 added note 56. 13.2.7 Added second centrifuge step 57. 13.2.13 added documentation 58. 13.3.1 added note to separate text 59. Section 13 -14 updated and clarified all steps 60. Updated Attachment to include T150 61. Update Attachment 2 and 3 cell confluency 62. Changed Table 1 and Table 2 numbering	21. Unnecessary 22. Ease of use 23. Reflect current practices 24. Ease of use 25. Reflect current practices 26. Clarification 27. Correction 28. Clarification 29. Clarification 30. Ease of use 31. Reflect reagents name 32. Reflect current practices 33. Ease of use 34. Clarification 35. Reflect current practices 36. Reflect current practices 37. Clarification 38. Clarification 39. Reflect current practices 40. Do not use 41. Clarification 42. Clarification 43. Clarification 44. Clarification 45. Clarification 46. Reflect current practices 47. Reflect current practices 48. Reflect current practices 49. Reflect current practices 50. Clarification 51. Clarification 52. Clarification 53. Ease of use 54. Clarification 55. Clarification 56. Reflect current practices 57. Clarification 58. Clarification 59. Clarification 60. Reflect current practices 61. Reflect current practices 62. Reflect location within SOP

Attachment 1: Lipofectamine 2000 Transfection

Cell Seeding Density by Flask Size (cells/flask)		
T-225	5-layer	8-Layer CELLdisk
21 x 10^6	84 x 10^6	189 x 10^6

Cell Confluency (Transfection – Day 2)	40-60%

Lipofectamine 2000:Media Preparation by Flask Size (per flask)		
Flask Size	Volume of Lipofectamine	Volume of Opti-MEM
T-225	247.5 µL	5.625 mL
5-layer	990 µL	22.5 mL
8-Layer CELLdisk	2228 µL	50.625 mL

Example Calculation: Transfect 40 T225 flasks plus calculate 2 additional flasks for overage.

42 flasks x 247.5 µL = 10.4 mL Lipofectamine Transfection Reagent
42 flasks x 5.625 mL = 236.25 mL Opti-MEM

Combine Transfection Reagent with Opti-MEM in a T75 Flask or a bottle.

DNA:Media Preparation by Flask Size (per flask)		
Flask Type	Amount of DNA	Volume of Opti-MEM
T225	112.5 µg	5.625 mL
5-Layer	450 µg	22.5 mL
8-Layer CELLdisk	1013 µg	50.625 mL

Example Calculation: Transfect 40 T225 flasks plus calculate 2 additional flasks for overage.

42 x 112.5 µg = 4,725 µg DNA
42 x 5.625 mL = 236.25 mL Opti-MEM

Using a 1000 µL pipette, add the correct concentrations of DNA to Opti-MEM and gently mix by inverting the tube several times.

Volume of Transfection Cocktail to add (per flask)	
Flask Type	Volume of Transfection Cocktail
T225	11.25 mL
5-Layer	46.0 mL
8-Layer CELLdisk	103.5 mL

Attachment 2: PEI Transfection

Cell Seeding Density by Flask Size (cells/flask)		
T-225	5-layer	8-Layer CELLdisk
35 x 10^6	160 x 10^6	252-270 x 10^6

Cell Confluency (Transfection – Day 2)	80-95%

PEI:Media Preparation by Flask Size (per flask)		
Flask Size	Volume of PEI	Volume of DMEM SF
T-225	337.5 µL	10 mL
5-layer	1350 µL	22.5 mL
8-Layer CELLdisk	3039 µL	50.625 mL
Example Calculation: Transfect 40 T225 flasks plus 2 additional flasks for overage. 42 flasks x 337.5 µL = 14.18 mL PEI Transfection Reagent 42 flasks x 10 mL = 420 mL DMEM SF Combine Transfection Reagent with DMEM SF in a T75 Flask or a bottle.		

DNA:Media Preparation by Flask Size (per flask)		
Flask Size	Amount of DNA	Volume of DMEM SF
T225	112.5 µg	10 mL
5-Layer	450 µg	22.5 mL
8-Layer CELLdisk	1013 µg	50.625 mL
Example Calculation: Transfect 40 T225 flasks plus 2 additional flasks for overage. 42 x 112.5 µg = 4,725 µg DNA 42 x 10 mL = 420 mL DMEM SF Using a pipette, add the DNA to DMEM SF and gently mix by inverting several times.		

Volume of Transfection Cocktail to add (per flask)	
Flask Type	Volume of Transfection Cocktail
T225	20 mL
5-Layer	46.0 mL
8-Layer CELLdisk	103.5 mL

Attachment 3: Transporter 5 Transfection

Cell Seeding Density by Flask Size (cells/flask)			
T-150	T-225	5-layer	8-Layer CELLdisk
25 x 10^6	35 x 10^6	160 x 10^6	252-270 x 10^6
Cell Confluency (Transfection – Day 2)		80-95%	

Transporter 5:Media Preparation by Flask Size (per flask)		
Flask Size	Volume of Transporter 5	Volume of DMEM 2%
T-150	225 µL	7 mL
T-225	337.5 µL	10 mL
5-layer	1350 µL	22.5 mL
8-Layer CELLdisk	3039 µL	50.625 mL

Example Calculation: Transfect 40 T225 flasks plus 2 additional flasks for overage.

42 flasks x 337.5 µL = 14.18 mL Transporter 5 Transfection Reagent
42 flasks x 20 mL + 20mL = 860 mL DMEM 2% split in two bottles of 420 mL

Combine Transfection Reagent with 420 mL of DMEM 2% in a Flask or a bottle.

DNA:Media Preparation by Flask Size (per flask)		
Flask Type	Amount of DNA	Volume of DMEM 2%
T150	75 µg	7 mL
T225	112.5 µg	10 mL
5-Layer	450 µg	22.5 mL
8-Layer CELLdisk	1013 µg	50.625 mL

Example Calculation: Transfect 40 T225 flasks plus 2 additional flasks for overage.

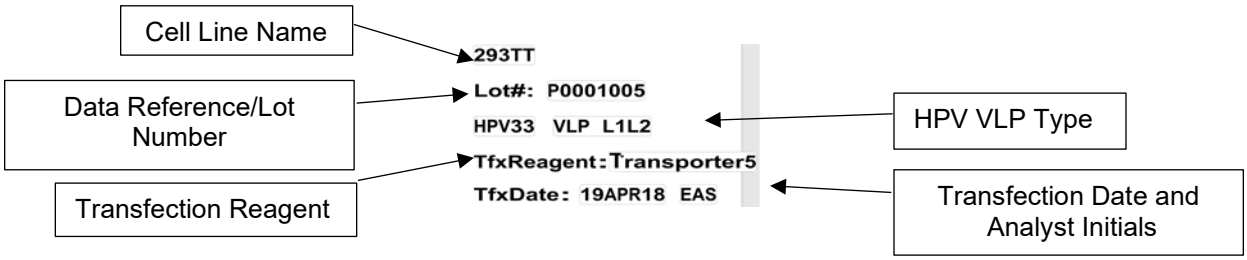
42 x 112.5 µg = 4,725 µg DNA

Using a pipette, add DNA to DMEM 2% and gently mix by inverting several times.

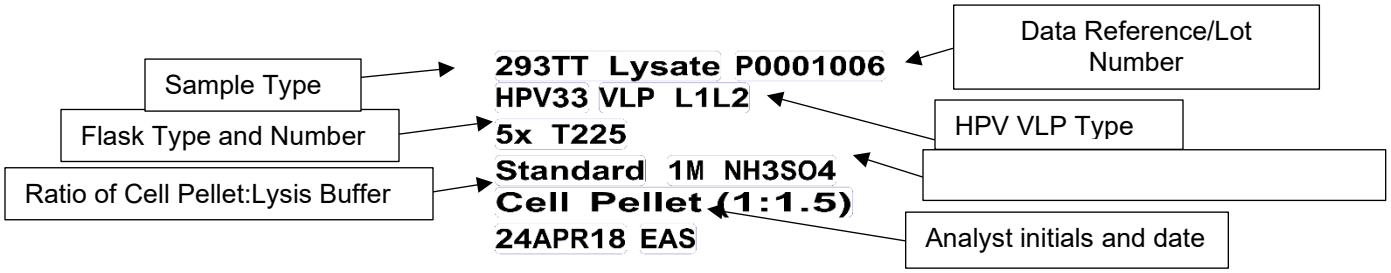
Volume of Transfection Cocktail to add (per flask)	
Flask Type	Volume of Transfection Cocktail
T150	28 mL
T225	20 mL
5-Layer	46.0 mL
8-Layer CELLdisk	103.5 mL

Attachment 4: Transfection Flask, Lysate Tube and Box, and Fraction Tube and Box Label Guidance

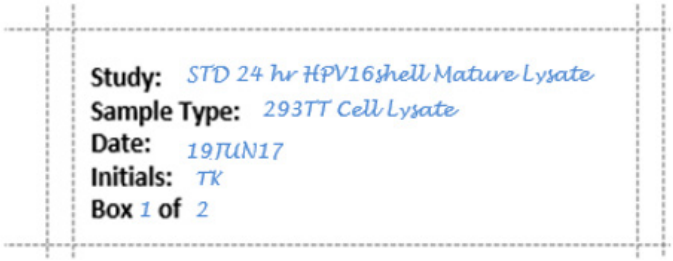
293TT TRANSFECTION (Tfx) FLASK LABEL



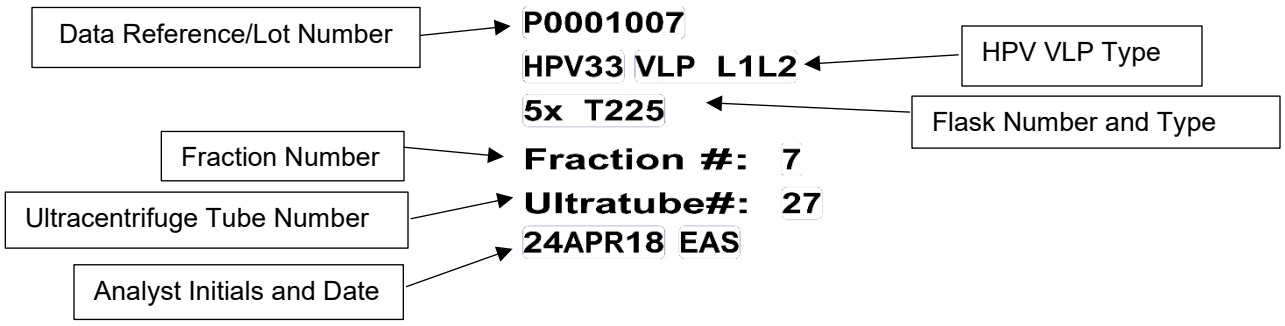
LYSATE TUBE LABEL



LYSATE TUBE BOX LABEL



FRACTION TUBE LABEL



FRACTION TUBE BOX LABEL

Study: *HPV16shellUltratube # XX-YY*
Sample Type: *OptiPrep*
Date: *19JUN17*
Initials: *TK*
Box *1* **of** *2*

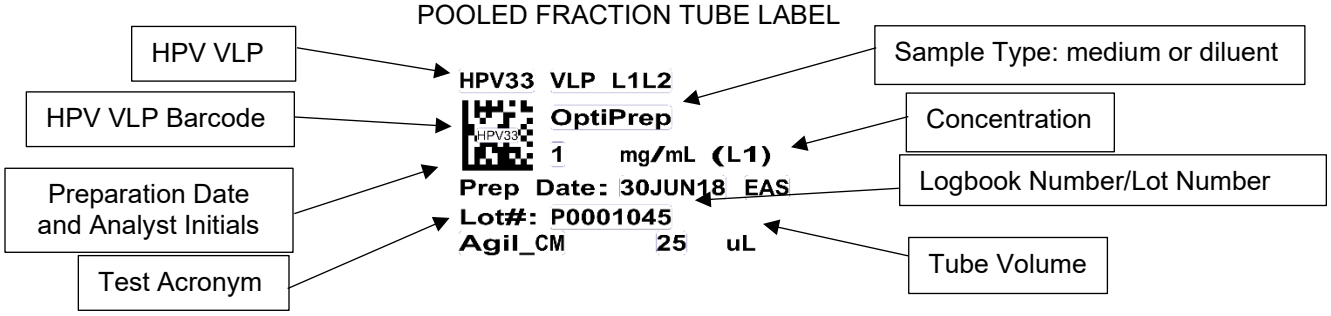
Study: *HPV16shellUltratube # XX-YY*
Sample Type: *OptiPrep*
Date: *19JUN17*
Initials: *TK*
Box *1* **of** *2*

Attachment 5: Suggested Aliquots for Testing, Pooled Fraction Tube and Box Label Guidance

SUGGESTED ALIQUOTS FOR POOLED FRACTION TESTING

Assay	Conc (mg/mL)	Volume (uL)
Combined Pooled Fraction	2.337	Various
Agilent and Coomassie (Agil_CM)	2.337	25
Bicinchoninic Protein Assay (BCA)	2.337	25
ELISA Specificity (ES)	2.337	25
Electron Microscopy (EM)	0.500	25
Dynamic Light Scattering (DLS)	2.337	150

POOLED FRACTION TUBE LABEL



POOLED FRACTION TUBE BOX LABEL

Study: HPV6 VLP L1L2
Sample Type: Optiprep
Date: 27AUG17
Initials: TK
Box 1 of 1
Lot#: H6_AB_27AUG17

Attachment 6: 20005.01: HEK293TT Transfection Form, Day 1-4

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Vaccine, Immunity and Cancer Directorate
Standard Operating Procedure Form

Form Title: HEK293TT Transfection Form, Day 1-4			
Document ID: 20005-01		Version:	5.0
Associated SOP: 20005		Effective Date:	07Sep21
Supersedes Version:	4.1	**Page 1 of 7**	

Day 1: Cell Preparation

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
CO_2 Incubator	☐ HSL_024 ☐HSL_023 ☐ HSL_026 ☐ HSL_027 ☐ Other:	
Inverted Microscope	☐ HSL_020 ☐ Other:	
2-8°C Refrigerator	☐ HSL_029 ☐ Other:	☐ N/A
Cellometer Auto 2000	☐ HSL_019 ☐ Other:	☐ N/A
Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	

Reagents

Reagent Name	Lot Number	Expiration Date
DPBS		
Trypsin-EDTA		
Vita Stain AOPI Staining Solution		

DMEM TF Preparation

Reagent Name	Lot Number	Expiration Date	Volume (mL) / Quantity
DMEM			
FBS			
NEAA			
Glutamax			
0.2 PES Filter unit			

Performed by/date:	

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Cell Culture Lot Number: _____

Working Passage Number: _____

Confluency: _____%

Cell Count

Count Number	Cell Concentration (x10^6 Cells/mL)	Viability (%) (≥80%)
1		☐ Pass ☐ Fail
2		☐ Pass ☐ Fail
3 ☐ N/A Row		☐ Pass ☐ Fail
4 ☐ N/A Row		☐ Pass ☐ Fail
Average Cell Count (x10^6 Cells/mL)		
Percent Difference (%) (≤ 25%)		
Secondary Average Cell Count (x10^6 Cells/mL) ☐ N/A Row		

Inoculation

Seeding Conc. of Flask (Cells/ Flask)	Total Volume Required (mL)	Volume of Cells (mL)	Volume of DMEM TF (mL)	Flask Type / # Prepared
☐ N/A Row				
☐ N/A Row				
☐ N/A Row				
☐ N/A Row				

Cell Culture Start Time: _____

Performed by/date:	
Reviewed by/date:	

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Day 2 – Transfection

Cell Culture Confluency: _____%

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
CO_2 Incubator	☐ HSL_024 ☐ HSL_023 ☐ HSL_026 ☐ HSL_027 ☐ Other:	
Inverted Microscope	☐ HSL_020 ☐ Other:	
NanoDrop	☐ HSL_036 ☐ HSL_068 ☐ Other:	☐ N/A
Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	

Reagents

Reagent Name	Lot Number	Expiration Date	Concentration
Transfection Reagent Description:			
HPV Plasmid DNA Description:		☐ N/A	

Media (Select One)

☐ N/A OPTI-MEM (Lipofectamine 2000)	Lot Number	Expiration Date

☐ N/A DMEM 2% or SF (T5 or PEI)	Reagent Name	Lot Number	Expiration Date	Volume (mL) / Quantity
	DMEM			
	☐ N/A FBS			
	NEAA			
	Glutamax			
	0.2 PES Filter Unit			

Performed by/date:	

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Nanodrop Data:
(Affix Nanodrop Data here)

Comments:

☐ N/A

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Transfection Cocktail Preparation

Transfection Reagent:Media		**DNA:Media**	
Reagent Name	Volume Used (mL)	Reagent Name	Volume Used (mL)
Transfection Reagent		HPV Plasmid DNA	
Transfection Media		Transfection Media	
Incubate for 5-10 minutes at RT			
Combine Transfection Reagent:Media and DNA:Media. Incubate for 20-30 minutes at RT			

Cell Culture Transfection

Volume of Transfection Cocktail / Flask (mL)	37°C Transfection Start Time*	48±2 hr 37°C Transfection End Time

* Transfection Incubation Start Time (based on the first Flask)

Post-Cell Culture Transfection DMEM TF Preparation

Reagent Name	Lot Number	Expiration Date	Volume (mL) / Quantity
DMEM			
FBS			
NEAA			
Glutamax			
0.2 PES Filter unit			

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

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Form Title: HEK293TT Transfection Form, Day 1-4

Document ID: 20005-01		Version:	5.0
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Day 4 – Cell Harvest

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐HSL_008 ☐ HSL_009 ☐ Other:	
CO_2 Incubator	☐ HSL_024 ☐ HSL_023 ☐ HSL_026 ☐HSL_027 ☐ Other:	
Inverted Microscope	☐ HSL_020 ☐Other:	
Sorvall Legend XTR Centrifuge	☐ HSL_033 ☐ Other:	
Water Bath	☐ HSL_010 ☐ Other:	
2-8°C Refrigerator	☐ HSL_029 ☐ Other:	☐ N/A
Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
-80°C Freezer	☐ HSL_022 ☐ Other:	
	☐ Freezer Inventory Updated	
	Shelf: **Rack:** ☐ N/A **Position:**	
	Date/Time stored:	

Reagents

Reagent Name	Lot Number	Expiration Date
DPBS		
Trypsin-EDTA		
5M NaCl		

Transfection Lysis Buffer

Reagent Name	Lot Number	Expiration Date	Volume Used (mL)
DPBS_MgCl_AA			
10% Brij58			
Benzonase			
Plasmid-Safe DNase			
1M Ammonium Sulfate			

Performed by/date:	

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Standard Operating Procedure Form

Form Title: HEK293TT Transfection Form, Day 1-4

Document ID: 20005-01		Version:	5.0
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Transfection Incubation End Time / Date*: _____
*- Based on the first Flask

VLP Maturation

37°C Incubation Start Time / Date	Invert Tubes within 1-2 hrs	37°C Incubation End Time / Date
	☐ Yes ☐ No, Add Comment	

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

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Attachment 7: 20005.02: HEK293TT Transfection Form, Day 5

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Form Title: HEK293TT Transfection Form, Day 5		
Document ID: 20005-02	Version:	5.0
Associated SOP: 20005	Effective Date:	07Sep21
Supersedes Version: 4.1	**Page 1 of 2**	

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
Eppendorf Centrifuge	☐ HSL_006 ☐ Other:	
Precision Balance	☐ HSL_015 ☐ Other:	
Ultracentrifuge	☐ HSL_001 ☐ Other:	
2-8°C Refrigerator	☐ HSL_029 ☐ Other:	☐ N/A
Rotor Used	☐ Sw 55 Ti ☐ Sw40 Ti	
Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
-80°C Freezer	☐ HSL_022 ☐ Other:	
	☐ Freezer Inventory Updated	
	Lysate **Shelf:**	**Rack:** ☐ N/A **Position:**
	In-process samples **Shelf:**	**Rack:** ☐ N/A **Position:**

Reagents

Reagent Name	Lot Number	Expiration Date
27% OptiPrep		
33% OptiPrep		
39% OptiPrep		
DPBS_0.8M		

Gradient Diffusion (For Information Only)

Start Time	End Time

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Form Title: HEK293TT Transfection Form, Day 5

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Ultracentrifuge Tube Information

Ultra-Centrifuge Tube Position	Ultra-Centrifuge Sequence #	Data Reference/Description of Lysate **Note**: If same lysate is used for multiple lines, select "Same as Tube:" and write lysate tube number.
☐N/A 1		
☐N/A 2		☐ Same as Tube:
☐N/A 3		☐ Same as Tube:
☐N/A 4		☐ Same as Tube:
☐N/A 5		☐ Same as Tube:
☐N/A 6		☐ Same as Tube:

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

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Attachment 8: 20005.03: Fraction Pool Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: Fraction Pool Form			
Document ID: 20005-03		Version:	5.0
Associated SOP: 20005		Effective Date:	07Sep21
Supersedes Version:	4.1	**Page 1 of 2**	

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
-80°C Freezer	☐ HSL_022 ☐ Other:	
	☐ Freezer Inventory Updated	
	Shelf: **Rack:** ☐ N/A **Position:**	

Number of VLP Product Tubes:		
VLP Sample Tubes:		
Assay	Concentration (mg/mL)	Volume (µL)
AgiI_CM		
BCA		
ES		
EM		
DLS		
☐N/A		
☐N/A		
Product Tube Label: (Affix one below as example)		

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Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

Vaccine, Immunity and Cancer Directorate
Standard Operating Procedure Form

Form Title: Fraction Pool Form

Document ID: 20005-03		Version:	5.0
Associated SOP: 20005		Effective Date:	07Sep21
Supersedes Version:	4.1	**Page 2 of 2**	

Fraction Pooling Table:
(Affix Pooling Table Here)

Performed by/date:	
Reviewed by/date:	

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