SOP Title: Ripcord pDNA Transfection in HEK293TT for Pseudoviruses (PsV) Production and Purification		
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Written by:		
Printed Name:	Title:	Signature/Date:

Approved by:		
Printed Name:	Title:	Signature/Date:
QA Approved by:		
Printed Name:	Title:	Signature/Date:

1. PURPOSE

1.1. The purpose of this procedure is to describe how to transfect Human Papillomavirus (HPV) plasmid DNA coding for capsid and plasmid DNA coding for a reporter into HEK293TT cells to produce Pseudovirions (PsV).

2. SCOPE

2.1. This procedure applies to the Vaccine, Immunity and Cancer Directorate laboratories.

2.2. This procedure will include the transfection of plasmid DNA into the HEK293TT cell line, PsV production, maturation, and purification of PsVs via a density-based gradient.

3. REFERENCES

3.1. 10009: General Record Review

3.2. 10010: Lot Number and Test Run Number Assignment

3.3. 15000: Waste Disposal at the Advanced Technology Research Facility

3.4. 15006: Reagent Preparation

3.5. 20001: 293TT Cell Culturing and Maintenance

3.6. 26000: Biosafety Cabinet (BSC) Use and Maintenance

3.7. 26001: Operation, Use and Maintenance of C02 Incubators

3.8. 26004: Use and Maintenance of the Cellometer Auto 2000

3.9. 26005: Use and Maintenance of a 2-8°C Refrigerator

3.10. 26007: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath

3.11. 26009: Use and Maintenance of Pipettes

3.12. 26012: Use and Maintenance of an Analytical & Precision Balance

3.13. 26013: Use and Maintenance of -20°C Freezer

3.14. 26015: Use and Maintenance of an Inverted Microscope

3.15. 26016: Operation, Use and Maintenance of the Water Purification System

3.16. 26017: Use and Maintenance of the Eppendorf Centrifuge

3.17. 26018: Use and Maintenance of NanoDrop Spectrophotometer

3.18. 26021: Use and Maintenance of the Optima XPN Ultracentrifuge System

3.19. 26030: Use and Maintenance of -80°C Freezers

3.20. 26033: Use and Maintenance of the Thermo Fisher Sorvall XTR Centrifuge

3.21. 30000_HPV Neutralization Assay for Titer Determination

3.22. 30010: Acrylamide Protein Gel Analysis of HPV Virus-Like Particles

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. DEFINITIONS

5.1. PEI – Polyethylenimine

5.2. PPB - Parts Per Billion

5.3. PsV – Pseudovirions

5.4. RT - Room Temperature

5.5. SDS - Safety Data Sheets

5.6. TOC – Total Oxidizable Carbon

5.7. Type II water - Pure/Analytical Grade, used for standard applications (Resistivity > 1 MΩ-cm and TOC ≤ 50 ppb).

6. REAGENTS, MATERIALS AND EQUIPMENT

6.1. Reagents

6.1.1. 10% Brij58 (15006: Section 23)

6.1.2. 27% OptiPrep (15006: Section 26)

6.1.3. 33% OptiPrep (15006: Section 27)

6.1.4. 39% OptiPrep (15006: Section 28)

6.1.5. 293TT VLP/PsV Transfection cell culture media (DMEM 10A) (15006: Section 21)

6.1.6. 5M NaCl (KD Medical, Cat # RGF-3270)

6.1.7. Dulbecco's Phosphate-Buffered Saline (DPBS) (Life Technologies, Cat # 14190-136)

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

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

6.1.10. Papillomavirus L1 or L1/L2 expression plasmid

6.1.11. "Pseudogenome" reporter plasmid (e.g., pYSEAP or pfwB)

6.1.12. HEK 293TT cells (20001)

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

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

6.1.15. PEI (15006: Section 34)

6.1.16. RNase A (Life Technologies, Cat # AM2286)

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

6.1.18. Trypsin-EDTA 0.05% (Life Technologies, Cat # 25300-054)

6.1.19. Type II water (Water Purification Systems, 26016)

6.2. Equipment

6.2.1. Class II Biosafety Cabinet (BSC)

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

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

6.2.4. Centrifuges (microcentrifuge, bench top, ultracentrifuge)

6.2.5. CO_2 Incubator

6.2.6. Inverted Light Microscope

6.2.7. NanoDrop

6.2.8. Pipettes (ranging from 2 µL to 1000 µL)

6.2.9. Precision Balance

6.2.10. Rotor SW40.1Ti, rated for > 200,000 x g

6.2.11. Rotor SW55Ti, rated for > 200,000 x g

6.2.12. Water Bath

6.2.13. Stand with clamp

6.2.14. Water Purification System

6.3. Consumables

6.3.1. 10 mL serological pipets (FNLCR Warehouse, Cat # 66401370 or equivalent)

6.3.2. 25 mL serological pipets (FNLCR Warehouse, Cat # 66401361 or equivalent)

6.3.3. 5 mL serological pipets (FNLCR Warehouse, Cat # 66401365 or equivalent)

6.3.4. 50 mL serological pipets (FNLCR Warehouse, Cat # 66401363 or equivalent)

6.3.5. 50 mL conical tubes (FNLCR Warehouse, Cat # 66401493 or equivalent)

6.3.6. 500 mL conical centrifuge tubes (Thomas Scientific, Cat # 8600A70 or equivalent)

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

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

6.3.9. BD 1 mL syringe with 25-gauge needle (FNLCR Warehouse, Cat # 66301465 or equivalent)

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

6.3.11. Media Storage Bottle 1 L (Thomas Scientific, Cat # 1743D15 or equivalent)

6.3.12. Nalgene 0.2 µM PES membrane 500 mL filter bottle (Thomas Scientific, Cat # 1234K58 or equivalent)

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

6.3.14. Siliconized 1000 µL pipet tips (Thomas Scientific, Cat # 7738E30 or equivalent)

6.3.15. Siliconized 200 µL pipet tips (Thomas Scientific, Cat # 7738E15 or equivalent)

6.3.16. T-150 Flask (Thomas Scientific, Cat # 9381J33 or equivalent)

6.3.17. T-225 Flask (Thomas Scientific, Cat # 9381M60 or equivalent)

6.3.18. Thinwall Polypropylene Tubes, 14 mL (Beckman Coulter, Cat # 331374)

6.3.19. Thinwall Polypropylene Tubes, 5 mL (Beckman Coulter, Cat # 326819)

6.3.20. Polystyrene Centrifuge Tubes, 50 mL (Thomas Scientific, Cat # 1216M90)

6.3.21. Pipette Tips

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, 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 Lipofectamine 2000.

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 0.5 – 2.0 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-10T Medium Preparation

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

Table 1: Transfection Media (**DMEM-10A**) Preparation

	DMEM-10A
Reagent Name	Volume 500 (mL)
DMEM	440
FBS	50
NEAA	5
Glutamax	5
0.2 µm PES Filter 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

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	1mL
DPBS_MgCl_AA (mL)	0.889 mL
10% Brij58	67 µL
RNase A	2.5 µL
1M Ammonium Sulfate (NH3SO4)	42 µL

9.3.2. Label with reagent name, 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: Transfection Lysis Buffer is required during the Day 4 step.

10. 293TT CELL PREPARATION (DAY 1)

Note: Enter pertinent information on "20006: 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. For T225 cell culture flasks, seed 21 x 10^6 293TT cells per flask in DMEM 10A in a total volume of 30 mL.

10.3. For 5-Layer cell culture flasks, seed 84 x 10^6 cells per flask in DMEM 10A in a total volume of 120 mL.

10.4. For 8-Layer CELLdisk culture flasks, seed 189 x 10^6 cells per flask in DMEM 10A in a total volume of 270 mL.

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

11. TRANSFECTION (DAY 2)

11.1. 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 has been reached

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

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

11.3. Confirm the concentration of DNA using the NanoDrop per “26018: Use and Maintenance of NanoDrop Spectrophotometers.”

11.4. Affix NanoDrop data to 20006-01 .

11.5. Prepare the Transfection Cocktail.

11.5.1. Prepare Transfection Reagent: Opti-MEM.

11.5.1.1. Prepare the Transfection Reagent: Opti-MEM mixture as shown in Table 2.

Note: “Transfection Reagent” can refer to either Lipofectamine 2000, PEI, or Transporter 5. Analyst may use any one of the three Transfection Reagents. Volumes and ratios are the same.

Table 2: Transfection Reagent: Opti-MEM ratio volumes

Flask Type	Transfection Reagent (per Flask)	Opti-MEM (per Flask)
T225	247.5 µL	5.625 mL
5-Layer	990 µL	22.5 mL
8-Layer CELLdisk	2228 µL	50.625 mL

Example:
Transfect 20 T225 Flasks

20 x 247.5 µL = 4.95 mL Transfection Reagent
20 x 5.625 mL = 112.5 mL Opti-MEM

Combine 4.95 mL Transfection Reagent with 112.5 mL Opti-MEM in a T75 Flask or bottle.

11.5.1.2. Incubate the Transfection Reagent: Opti-MEM mixture for 5-10 minutes at room temperature.

Note: Do not allow the Transfection Reagent to sit in Opti-MEM for longer than 25 minutes.

11.5.2. Prepare DNA: Opti-MEM.

11.5.2.1. Prepare the DNA: Opti-MEM mixture as shown in Table 3. Invert to mix.

Table 3: DNA: Opti-MEM ratio volumes

Flask Type	HPV DNA (per Flask)	Reporter DNA (per Flask)	Opti-MEM (per Flask)
T225	56.25 µg	56.25 µg	5.625 mL
5-Layer	225 µg	225 µg	22.5 mL
8-Layer CELLdisk	506.5 µg	506.5 µg	50.625 mL

11.5.3. Add the Transfection Reagent: Opti-MEM mixture to the DNA: Opti-MEM mixture into the appropriate-sized flask or bottle. (This is now called **Transfection Cocktail**). Invert to mix.

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

11.6. Remove the 293TT flasks from the incubator and place in the BSC. Leave conditioned media on cells.

11.7. Gently mix the Transfection Cocktail and add volume to flask per Table 4.

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

11.7.2. 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

Table 4: Volume of Transfection Cocktail to add to each flask

Flask Type	Transfection Cocktail (per Flask)
T225	11.5 mL
5-Layer	46.0 mL
8-Layer CELLdisk	103.5 mL

11.8. Label each flask with cell type, working passage number, transfection date, transfection reagent, plasmid name, and analyst initials.

11.9. Incubate the cells in a 37±2°C, 5±2% CO_2 incubator for 5-6 hours.

Note: Use the start time of the first flask transfected on 20006-01.

Note: The start time of the Transfection at 37±2°C (Step 11.9) is also the start time of the 48 ± 2-hour Transfection Incubation at 37±2°C (Step 11.11).

11.10. Remove flasks from the incubator and place in the BSC. Remove the media using a sterile serological pipet, or by decanting, into a waste container. Add room temperature **DMEM-10A** to each flask per Table 5.

Table 5: Volume of DMEM-10A to add to each flask

Flask Type	DMEM-10A (per flask)
T225	45 mL
5-Layer	180 mL
8-Layer CELLdisk	405 mL

11.11. Incubate transfected cells in the 37°C, 5% CO_2 incubator for 48 ± 2 hours.

12. CELL HARVEST (DAY 4)

12.1. Place flasks in the BSC. Remove the media using sterile serological pipet, or by decanting, into an appropriately sized container "**A.**"

12.2. Gently wash attached cells with DPBS.

12.2.1. Add volume of DPBS per Table 6 to each flask.

Table 6: Volume of DPBS to add to each flask

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

12.2.2. Gently wash attached cells by rocking flasks 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 each flask per Table 7. Gently rock flasks to distribute trypsin evenly over cells and incubate for 3-5 minutes in the 37±2°C, 5±2% CO_2 incubator. Prolonged exposure to trypsin approaching 25 minutes is toxic to cells.

Table 7: Volume of Trypsin to add to each flask

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

12.4. Place flasks into the BSC. Wash flasks with media from container "**A**" to collect detached cells and add into container "**B**." Use volumes listed in Table 6.

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

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

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

12.7. Centrifuge balanced tubes at 300 ± 20 x g for 10 ± 1 minutes at 20 ± 5°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 the cells via swirling the tube, finger tapping or using a serological pipet. Cells may be consolidated into a 50mL conical tube.

12.10. Centrifuge bottles or tubes at 300 ± 20 x g for 10 ± 1 minutes at 20 ± 5°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 bottle or 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 9.3) (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 1 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.

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

12.18. 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.

Note: DO NOT add salt. Adding salt at this step would solubilize the cellular DNA. This would ruin the preparation due to extreme viscosity.

12.19. Proceed to VLP purification (Section 13, Day 5) for immediate processing or transfer tubes to labeled box (Attachment 4) and freeze tubes in -65°C to -90°C freezer for subsequent purification.

13. GRADIENT AND PURIFICATION (DAY 5)

Note: Enter pertinent information on "20006-02: HEK293TT Transfection Form, Day 5."

13.1. Ultracentrifuge Preparation

13.1.1. Turn on the 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 8. Confirm settings for rotor type, speed, tube size and temperature per 26021.

Table 8: Ultracentrifuge Gradient Program Settings

Program Name	Rotor Type	Tube P/N / Size	Volume of Each Gradient to Use (µL)	Rotor Speed	Temperature	Length of time (hour : min)
HPV_PsV	SW 55 Ti	326819 / 5 mL	1200	303,800 x g	16°C	03:30
HPV_PsV_SW 40	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 clean cannula, add 27% Opti-Prep to the bottom of the tube.

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 the 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 2-8°C per "26017: Use and Maintenance of the Eppendorf Centrifuge."

13.2.5. If lysates are frozen, while gradient is diffusing, remove the lysates from step 12.18 from -65°C to -90°C freezer and thaw on wet ice. Once lysates are completely thawed, invert tubes gently 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 the lysate by centrifuging at 10,000 ± 1000 x g at 4 ± 2°C for 10±1 minute 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.

13.2.8. Place Supernatant tubes into the BSC. Remove the clarified supernatant and transfer to 1.5 mL siliconized tubes or 50 mL polystyrene tube labelled "Supernatant." Store "Supernatant" tube on wet ice until gradient has fully diffused.

13.3. Ultracentrifugation

13.3.1. Inside the BSC, carefully add the collected supernatant to the top of the Opti-Prep gradient using a siliconized pipet tip.

Note: Pipette supernatant slowly to ensure the gradient is not disturbed.

13.3.2. Fill all ultratubes to approximately 4 mm from the top of the tube to prevent collapse during ultracentrifugation. Cap ultratubes and carefully transfer ultratubes into ultracentrifuge buckets.

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

Notes: Pair Bucket 1 with Bucket 4, Bucket 2 with Bucket 5, and Bucket 3 with Bucket 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 the rotor into ultracentrifuge, and select the appropriate program corresponding to Table 8. Start ultracentrifuge run.

Note: To avoid disturbing the gradient, use minimal brake.

13.3.8. Once the 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 ultratube processed: fractions 1-10, plus 20 µL aliquots of fractions 1-10.

Note: See Attachment 1 for label guidance.

13.4.2. Place tubes into the 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 25-gauge 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 9 via gravity-based dripping. Place fractions into ten labeled tubes, fractions 1-10 in order, from step 13.4.1.

Table 9: 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)
SW 55 Ti	326819 / 5 mL	400	200
SW40 Ti	331374 / 14 mL	1000-1300	300

13.4.4. Gently mix the 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 PsV fractions at -65°C to -90°C in a box labeled per Attachment 1. Store the 20 µL aliquots at -65°C to -90°C in a separate box labeled per Attachment 1.

14. INFECTIVITY ASSAY FOR FRACTIONS COLLECTED

14.1. Prepare Assay Plates (flat-bottom 96-well plates) containing 293TT cells according to “30000: HPV Neutralization Assay for Titer Determination”.

14.2. Thaw the PsV fractions on wet ice inside a biological safety cabinet.

14.3. Prepare a set of round-bottom plates, and label the lids with the date, batch# (Example: infectivity test #1), HPV type, and the assigned number for the polyallomer tube (Example Tube 1), being tested on this **First** set of plates (see Figure 1 as an example).

Figure 1. 96 well plate schematic of dilutions of PsV fractions.

		empty	empty	Fraction (F)1	F2	F3	F4	F5	F6	F7	F8	empty	empty	
		1	2	3	4	5	6	7	8	9	10	11	12	
Tube 1	A													
	B			20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL			1:10 diltuion
	C			20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL			1:100 dilution
	D													
Tube 2	E													
	F			20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL			1:10 diltuion
	G			20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL			1:100 dilution
	H													

14.3.1. In this example, eight different fractions that were collected from two different polyallomer tubes are serially diluted: 1:10 to 1:100 (Rows B and C for Tube 1, and Rows F and G for Tube 2).

14.3.2. Fractions 1 to 8 from each tube was dispensed into Columns 3 to 10 in a numerical order. Numbers in black indicate the volume of neat PsV solution (Rows B and F), or volume of 1:10 diluted PsVs (Rows C and G). The numbers in purple indicate the volume of Assay Media.

14.4. Prepare 1:10 dilution for PsV fractions (Example: 20 µL of neat PsV solution + 180 µL of Assay Media) in a round-bottom 96-well plate.

14.5. In the next row of the same round-bottom 96-well plate, perform another 1:10 dilution by transferring 20 µL of the 1:10 diluted PsV solution into 180 µL of Assay Media. The final dilution factor in these wells will be 1:100 (Figure 1).

14.6. In a separate, **Second** set of round-bottom 96-well plates, dispense 180 µL of Assay Media in Row A for the number of fractions being tested (see Figure 2 for example), and 150 µL of Assay Media in all the other wells in the corresponding columns (see Figure 2 for example).

Figure 2. Assay Master Plate set-up.

	Assay Media	Assay Media	Fraction (F)1	F2	F3	F4	F5	F6	F7	F8	Assay Media	Assay Media	
	1	2	3	4	5	6	7	8	9	10	11	12	Diltuion factor
A	200 uL	200 uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	20 uL + 180uL	200 uL	200 uL	1,000
B	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	4,000
C	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	16,000
D	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	64,000
E	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	256,000
F	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	1,024,000
G	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	4,096,000
H	200 uL	200 uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	50 uL + 150uL	200 uL	200 uL	16,384,000

14.6.1. Transfer 20 µL of the 1:100 diluted PsV solution (in black) from the **First** round-bottom plate (Rows C or G in Figure 1) into the corresponding Columns in Row A; this will produce a final dilution factor of 1:1000.

14.6.2. After mixing, transfer 50 µL of the 1:1000 diluted PsV into the next row, and mix. Repeat this process until reaching Row H.

Note: One plate per fractions from one polyallomer tube. This layout will also serve a plate map.

14.6.3. After mixing the samples in Row H, leave all volumes of solution in the wells, and put the lid of the plate on and set it aside inside the hood.

14.6.4. In Columns 1, 2, 11, and 12, 200 µL of Assay Media is dispensed. These wells will serve as controls.

14.6.5. Columns 3 to 10 are used to test serially diluted PsV from fractions collected from one polyallomer tube (F1 to F8).

14.7. Repeat the 4-fold serial dilution for each plate prepared for each polyallomer tube.

14.8. In a Third set of round-bottom 96-well plates, dispense 25 µL of Assay Media to into all the wells (see Figure 3a).

Figure 3a. Third set of round-bottom 96-well plates .

	Control wells	Control wells	Fraction (F)1	F2	F3	F4	F5	F6	F7	F8	Control wells	Control wells
	1	2	3	4	5	6	7	8	9	10	11	12
A	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
B	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
C	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
D	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
E	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
F	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
G	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL
H	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL	25 uL

14.9. From the **Second** round-bottom 96-well plate, transfer 100 µL of serially diluted PsVs or Assay Media into the corresponding wells in the **Third** set of a round-bottom 96-well plates that contain 25 µL of Assay Media.

14.10. Incubate the round-bottom 96-well plates with Assay Media and diluted PsVs for 40-80 minutes at 2-8°C. Record Start and End Time in Batch Information Forms for Infection Assay.

14.11. After the incubation, take out the round-bottom 96-well plates from the 2-8°C containment, as well as the Assay Plates (flat-bottom 96-well plates) containing 293TT cells from the 37 ± 2°C CO_2 incubator.

14.12. Using a multichannel pipet, transfer 100 µL (of 125 µL) of the Assay Media/diluted PsV solutions to appropriate wells in the Assay Plate (flat-bottom 96-well plates) that contains 293TT cells.

14.13. When finished dispensing, incubate Assay Plates for this HPV type in the same 37 ± 2°C CO_2 tissue culture incubator for 3 days (70-74 hours).

14.14. Perform Day2: HARVEST and DAY 3: CHEMILUMINESCENCE - SEAP SUBSTRATE DEVELOPMENT according to “30000: HPV Neutralization Assay for Titer Determination”.

14.15. Data analysis

14.15.1. In the exported Excel data file, RLUs of each well of the plates that were tested will be present (see below).

Tube 1													
	No-Virus control		Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	No-Virus control		
	1	2	3	4	5	6	7	8	9	10	11	12	dilution factor
A	517	547	1,094	8,756	2,649,598	2,709,292	2,059,899	1,401,705	986,734	1,010,914	1,680	512	1,000
B	389	547	1,128	7,130	2,519,499	2,473,214	1,199,568	617,482	395,748	374,530	1,419	557	4,000
C	439	493	848	4,543	2,087,291	2,043,347	402,366	180,470	95,838	96,380	823	557	16,000
D	443	542	941	4,198	1,217,150	1,029,333	141,734	43,077	26,471	22,100	591	616	64,000
E	498	562	857	1,961	414,394	421,603	30,975	12,452	9,072	7,623	606	557	256,000
F	434	660	715	1,252	150,490	126,133	12,738	1,735	1,636	2,281	478	527	1,024,000
G	389	453	527	769	40,126	40,697	6,002	1,971	577	1,237	429	375	4,096,000
H	355	567	473	596	10,816	10,141	4,277	675	517	596	370	424	16,384,000

14.15.2. Examine the RLUs in each fraction. A general trend should be that with each successive fraction tested, RLU should increase, then, it will decline in later fractions (i.e., a bell-shaped curve).

14.15.3. General guideline is to choose RLUs of at least 100,000 -250,000 in fraction(s) and consistent dilution factor between fraction(s) that were collected from OptiPrep ultracentrifugation tubes.

14.15.4. In the above example, fractions 3-4 were expected to contain the PsV particles of interest. This preparation of PsV particles may be diluted to approximately 1 x 10^6 fold, and still obtain 100,000 to 150,000 RLUs (see the green highlights below).

Tube 1													
	No-Virus control		Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	No-Virus control		
	1	2	3	4	5	6	7	8	9	10	11	12	dilution factor
A	517	547	1,094	8,756	2,649,598	2,709,292	2,059,899	1,401,705	986,734	1,010,914	1,680	512	1,000
B	389	547	1,128	7,130	2,519,499	2,473,214	1,199,568	617,482	395,748	374,530	1,419	557	4,000
C	439	493	848	4,543	2,087,291	2,043,347	402,366	180,470	95,838	96,380	823	557	16,000
D	443	542	941	4,198	1,217,150	1,029,333	141,734	43,077	26,471	22,100	591	616	64,000
E	498	562	857	1,961	414,394	421,603	30,975	12,452	9,072	7,623	606	557	256,000
F	434	660	715	1,252	150,490	126,133	12,738	1,735	1,636	2,281	478	527	1,024,000
G	389	453	527	769	40,126	40,697	6,002	1,971	577	1,237	429	375	4,096,000
H	355	567	473	596	10,816	10,141	4,277	675	517	596	370	424	16,384,000

15. FRACTION POOLING

15.1. Pool fractions selected by Scientific Manager or designee based on in-process testing results from 30000 SOP and Infectivity assay (Step 14).

15.1.1. Affix pooled fraction selection table to “20006-03: Fraction Pool Form;” confer with Scientific Manager or designee.

15.2. Thaw selected 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.

15.3. Label each siliconized tube according to Attachment 2.

Note: Lot numbers are assigned based on 10010 SOP.

15.4. Aliquot neat pooled PsV particles in 1.5 mL screw-cap conical siliconized tubes, and store in a -80 freezer in a 2-inch sample box. Volume of aliquots is generally 50 µL per vial.

15.5. Record number of aliquots into Freezer Inventory.

15.6. Affix pooled fraction selection table to “20006.03: Fraction Pool Form.”

15.7. Repeat Infectivity Assay at least two times to ensure that a similar level of infection (RLUs) can be obtained.

15.8. Once confident that a dilution factor chosen can provide the ideal levels of RLUs, perform qualification of the PsV particles using serum samples.

16. ATTACHMENTS

16.1. Attachment 1: Transfection Flask, Lysate Tube and Box, and Fraction Tube and Box Label Guidance

16.2. Attachment 2: Pooled Fraction Tube and Box Label Guidance and Suggested Aliquots for Testing

16.3. Attachment 3: 20006-01: HEK293TT Transfection Form, Day 1-4

16.4. Attachment 4: 20006-02: HEK293TT Transfection form, Day 5

16.5. Attachment 5: 20006-03: Fraction Pool Form

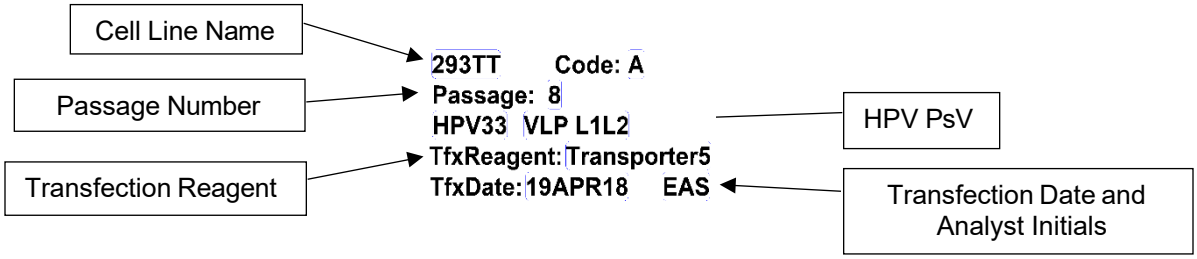
17. REVISION HISTORY

Version	Change	Reason

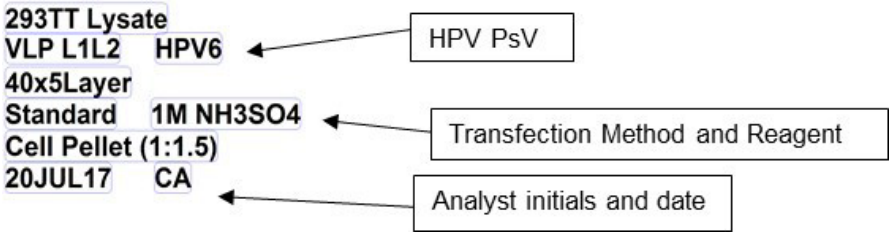
1.0	1. Updated document ID for SOP, HSL_LAB_013 converted to 20006. 2. Modeled changes after changes to 20005. 3. Updated formatting, forms as separate documents. 4. New form, 20006-03. 5. New Attachment 2. 6. Moved volume information into tables throughout document. 7. Added references to procedures throughout document. 8. Removed HSL_GL_002, HSL_GL_003, HSL_GL_004, HSL_GL_007, HSL_GL_008, HSL_GL_009, HSL_GL_010, HSL_EQ_009. 9. Added 10009, 10010, 15000, 15006, 20001, 26000, 26001, 26004, 26005, 26007, 26009, 26012, 26013, 26015, 26016, 26017, 26018, 26021, 26030, 26033, 30000, 30010. 10. Added stand and clamp to equipment. 11. Added Type II water to materials section. Added procedure references to source materials. 12. Removed ATRF, HPV, HSL, SOP from definitions. Added RT, (test acronyms) to definitions. 13. Section 8: Lysis Buffer volume table added. 14. Added step to affix Nanodrop data. 15. Revised Transfection mixture section: clarified names (added Transfection Cocktail), formatting. 16. Add infectivity assay for collected fractions 17. Added Pooling section.	1. Reflect Current Document ID structure per SOP 10000. 2. Same process as 20005 except for lysis buffer. 3. Consistency between procedures. 4. Capture pooling, reflect current practice. 5. Reflect current labelling practice. 6. Clarification, ease of use. 7. Clarification. 8. Removed procedures not listed in body of document. Added procedures listed in body of document. 9. Updated and included all relevant SOPs. 10. Used in procedure. 11. Type II water mentioned in body of document. Clarification. 12. ATRF and HPV acronyms included earlier in procedure. HSL and SOP not listed in procedure. RT, (test acronyms) listed on form and attachment. 13. Flexibility in volume preparation. 14. Clarification, ease of use. 15. Clarification. 16. Reflect current practice. 17. Reflect current practice.

Attachment 1: Transfection Flask, Lysate Tube and Box, and Fraction Tube and Box Label Guidance

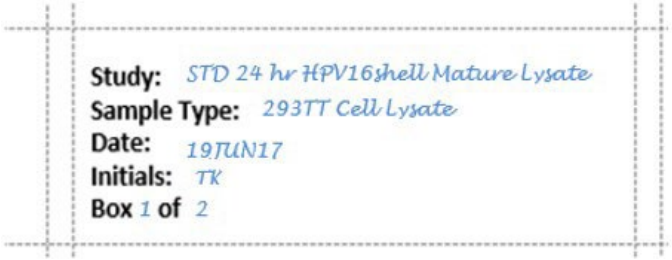
293TT TRANSFECTION (Tfx) FLASK LABEL



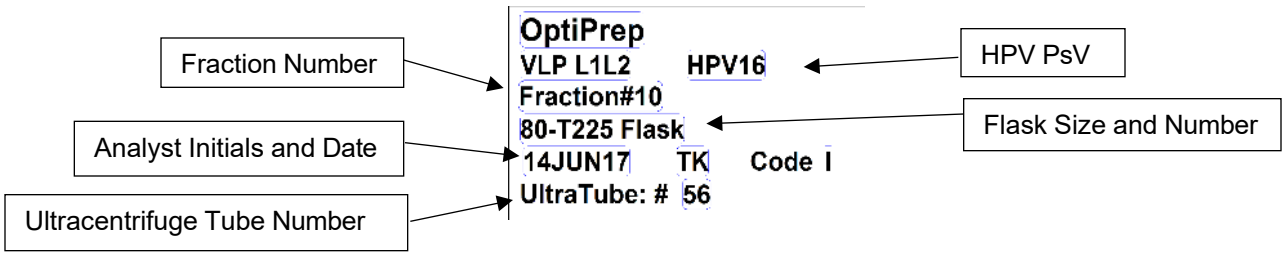
LYSATE TUBE LABEL



LYSATE TUBE BOX LABEL



FRACTION TUBE LABEL

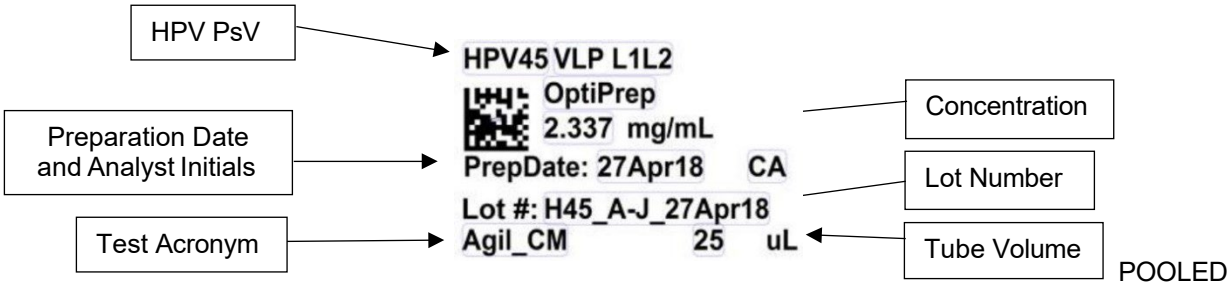


FRACTION TUBE BOX LABEL

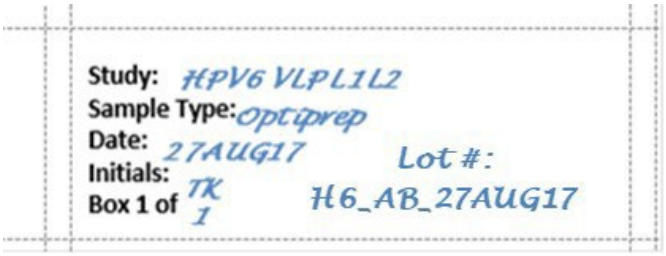
Study: *HPV16shell Ultratube # XX-YY*
Sample Type: *OptiPrep*
Date: *19JUN17*
Initials: *TK*
Box *1* **of** *2*

Attachment 2: Pooled Fraction Tube and Box Label Guidance and Suggested Aliquots for Testing

FRACTION TUBE LABEL



POOLED FRACTION TUBE BOX LABEL



SUGGESTED ALIQUOTS FOR POOLED FRACTION TESTING:

Assay	Volume (µL)
Combined Pooled Fraction	Various
Infectivity Assay	50

Attachment 3: 20006-01 HEK293TT Transfection Form, Day 1-4

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form
Form Title: HEK293TT Transfection Form, Day 1-4		
Document ID: 20006-01	Version:	1.0
Associated SOP: 20006	Effective Date:	27 Dec 21
Supersedes Version: New	**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:	
Cellometer Auto 2000	☐ HSL_019 ☐Other:	☐N/A
Pipette: µL	PIP_	

Reagents

Reagent Name	Lot Number	Expiration Date
DMEM 10A		
DPBS		
Trypsin-EDTA		
Vita Stain AOPI Staining Solution		

Cell Culture Lot Number: _____

Working Passage Number: _____

Confluency: _____

Cell Count

Count 1 (Cells/ mL)	Viability 1 (%)	Count 2 (Cells/ mL)	Viability 2 (%)	Average Count (Cells/ mL)	Percent Difference (%)
	☐Pass ☐Fail		☐Pass ☐Fail		
☐N/A Row	☐Pass ☐Fail		☐Pass ☐Fail	(Counts1-4)	N/A

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Inoculation

Seeding Conc. of Flask (Cells/ Flask)	Total Volume Required (mL)	Volume of Cells (mL)	Volume of DMEM 10A (mL)	Flask Type/ # Prepared
☐N/A Row				
☐N/A Row				
☐N/A Row				
☐N/A Row				

Cell Culture Start Time: _____

Comments:

☐ N/A

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 ☐Other:	
Pipette: µL	PIP_	
☐N/A Pipette: µL	PIP_	

Reagents

Reagent Name	Lot Number	Expiration Date	Concentration
DMEM 10A			
Opti-MEM			
Transfection Reagent Description:			
HPV Plasmid DNA Description:			
Reporter Plasmid DNA Description:			
Comments: ☐N/A			

Performed by/date:	
Reviewed by/date:	

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HPV Serology Laboratory
Standard Operating Procedure
Form

Form Title: HEK293TT Transfection Form, Day 1-4

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NanoDrop 1000 Data:
(Affix NanoDrop 1000 Data here)

Performed by/date:	
Reviewed by/date:	

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Transfection Cocktail Preparation

Transfection Reagent: Opti-MEM	
Reagent Name	**Volume Used (mL)**
Transfection Reagent	
Opti-MEM	
Incubate for 5-10 minutes at RT	

DNA: Opti-MEM	
Reagent Name	**Volume Used (mL)**
HPV Plasmid DNA	
Reporter Plasmid DNA	
Opti-MEM	
Incubate for 5-10 minutes at RT	

Combine Transfection Reagent: Opti-MEM and DNA: Opti-MEM. Incubate for 20-30 minutes at RT

Cell Culture Transfection

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

Post-Transfection Incubation Start Time / Date: _____

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

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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_	
-80°C Freezer	☐ HSL_022 ☐ Other:	
	☐ Freezer Inventory Updated	
	Shelf:	**Rack:**
	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			
RNase A			

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Form Title: HEK293TT Transfection Form, Day 1-4			
Document ID: 20006-01		Version:	1.0
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VLP Maturation

37°C Incubation Start Time / Date	37°C Incubation End Time / Date

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

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Attachment 4: HEK293TT Transfection Form, Day 5

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: HEK293TT Transfection Form, Day 5			
Document ID: 20006-02		Version:	1.0
Associated SOP: 20006		Effective Date:	27 Dec 21
Supersedes Version:	New	**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:	
-80°C Freezer	☐ HSL_022 ☐Other:	
2-8°C Refrigerator	☐ HSL_029 ☐Other:	
Rotor Used	☐ Sw 55 Ti ☐ Sw40 1 Ti	N/A
☐N/A Pipette: µL	PIP_	
☐N/A Pipette: µL	PIP_	

Reagents

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

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:

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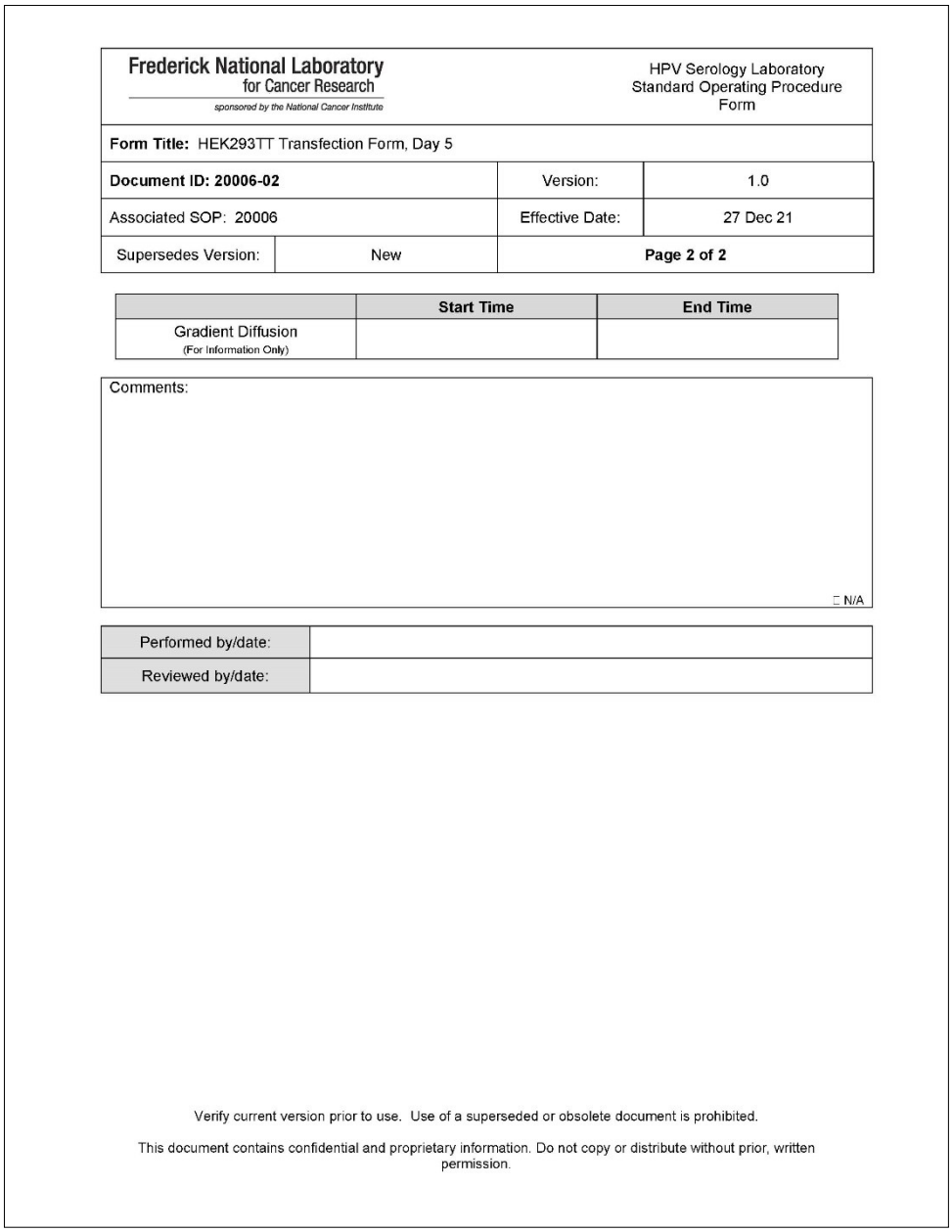

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

HPV Serology Laboratory
Standard Operating Procedure
Form

Form Title: HEK293TT Transfection Form, Day 5

Document ID: 20006-02	Version:	1.0
Associated SOP: 20006	Effective Date:	27 Dec 21
Supersedes Version: New	**Page 2 of 2**	

	Start Time	**End Time**
Gradient Diffusion (For Information Only)		

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

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Attachment 5: 20006-03 Fraction Pool Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: Fraction Pool Form			
Document ID: 20006-03		Version:	1.0
Associated SOP: 20006		Effective Date:	27 Dec 21
Supersedes Version:	New	**Page 1 of 2**	

Equipment

Equipment Name	Equipment ID	Calibration Due Date
-80°C Freezer	☐ HSL_022 ☐ Other:	
	☐ Freezer Inventory Updated	
	Shelf:	**Rack:**

Fraction Pooling Table:

(Affix Pooling Table Here)

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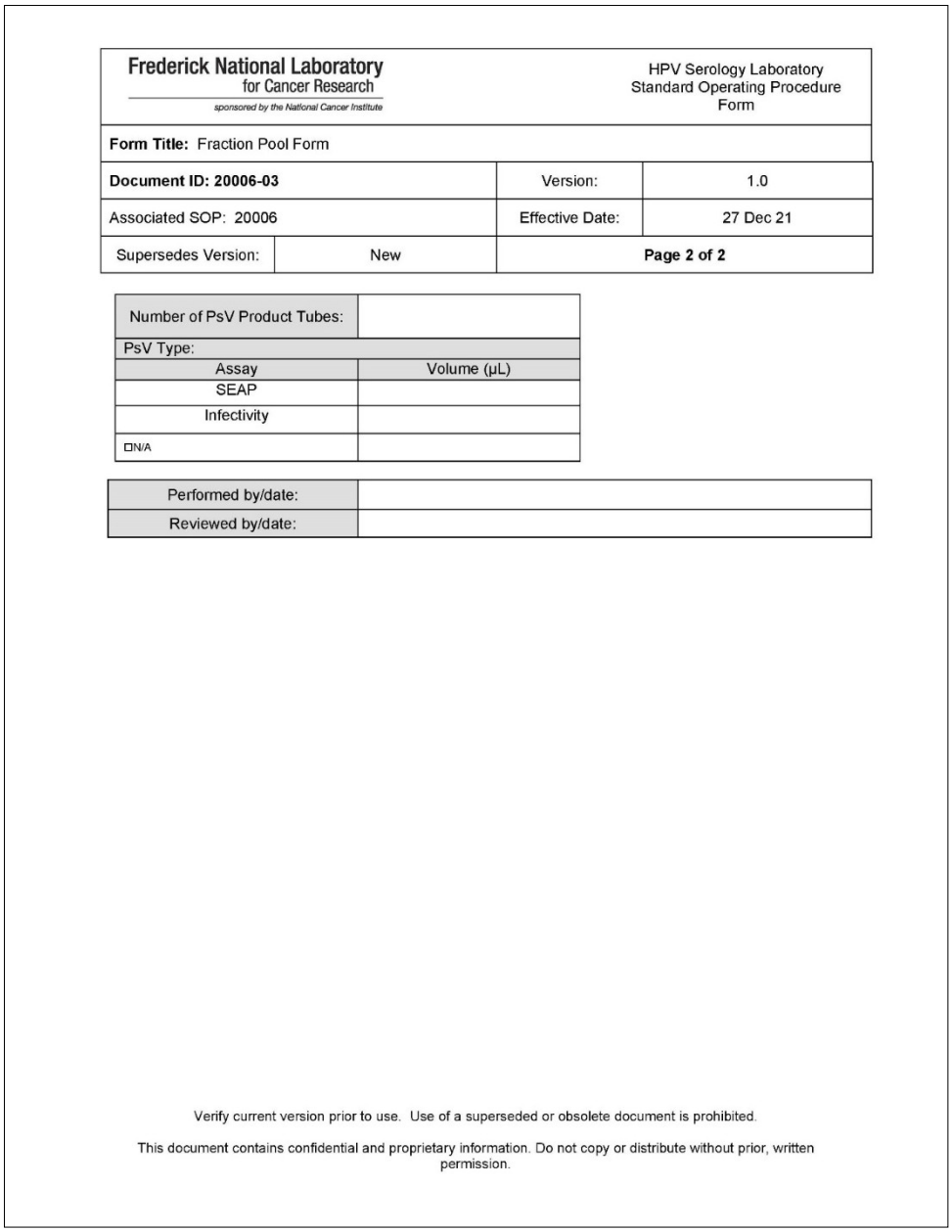

Frederick National Laboratory for Cancer Research
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HPV Serology Laboratory
Standard Operating Procedure
Form

Form Title: Fraction Pool Form

Document ID: 20006-03		Version:	1.0
Associated SOP: 20006		Effective Date:	27 Dec 21
Supersedes Version:	New	**Page 2 of 2**	

Number of PsV Product Tubes:	

PsV Type:

Assay	Volume (µL)
SEAP	
Infectivity	
☐N/A	

Performed by/date:	
Reviewed by/date:	

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