

STS-8 - CFES
KIDNEY CELL SAMPLE PREPARATION
(Samples 3 and 4)

Cell Wash and Preparation

3 4 Caution: Check valve 2 - closed (*using arrow*)

- 1) Place valve 1 in position 1
- 2) Expel contents of C syringe (to 3 ml mark) into syringe 1
- 3) Place valve 1 in position 2
- 4) Input 15 ml of wash from syringe 2 to syringe C
- 5) Shake tray to mix contents of syringe C
- 6) Expel contents of syringe C (to 3 ml mark) back into syringe 2

Enzyme Treatment

- 7) Turn valve 1 to position 3
- 8) Input 5 ml of enzyme from syringe 3 to syringe C, shake tray five seconds *crit.*
to mix contents of syringe C and allow to stand for 10 seconds *critical*
- 9) Expel contents of syringe C (to 3 ml mark) back into syringe 3
and allow C to remain undisturbed for 20 minutes (set timer for 18 min.) *critical*

Caution: To avoid backflow during next steps do not release pressure on side port syringe until valve 2 (side port valve) is closed.

SP

Cell Harvest

- 10) Backwash filter in syringe C by adding 1 ml from syringe 3
- 11) Place valve ¹ in position 4 (*closed*)
- 12) Open valve 2 (between syringe ⁴ and side port) *of syrg. C*
- 13) Input 5 ml of serum/buffer from syringe ⁴ to syringe C and immediately *from syrg. C*
- 14) Close valve 2 (keeping pressure on syringe 4 plunger) *SP*
- 15) Shake tray five seconds to mix contents of syringe C and expel 2 ml of liquid back into syringe 3

Caution

15) Please val 1 in pos. 3

Transfer to CFES Syringe

*sterilizing the
syringe*
57

- 16) Turn valve 1 to position 5 (flow into CFES syringe)
- 17) Expel remaining volume of cells from syringe C (to 3 ml mark) into CFES syringe → *MARKING*
- 18) Turn valve 1 to position 4 (closed) then open valve 2 (side port valve) *(INSERT CAUTION)*
- 19) Add ⁶ ml more from side syringe (4) to syringe C; immediately close valve 2 (^{CAPS}keeping pressure on syringe 4)
- 20) Shake tray to mix contents of syringe C
- 21) Turn valve 1 back to position 5
- 22) Expel contents of syringe C ^{into CFES syr. to specified volume} ~~into CFES syringe~~ → *MARKING*
- 24) Turn valve 1 to position 4 (closed)

Sterile Transfer to CFES

~~NOTE: ALWAYS USE ASEPTIC TECHNIQUE~~

- 23) Remove CFES syringe from CTA (pull straight off needle)
- 24) Move to CFES, then using aseptic technique remove sterile rubber cap on CFES syringe and insert immediately onto CFES input needle

NOTE: CFES Syringe should contain at least 10 ml; if not, repeat steps 16-20 to obtain at least 10 ml in CFES syringe.

1721

*make this
note
has to do with
pumping/bumping
over to gas,
syr. plunger -*

CFES
SAMPLES 3 AND 4 PREP

CELL WASH / PREP

3

4

• CAUTION.

✓ V2 VLV CL

1. VI vlv - POS 1
2. Contents of C (to 3ml mark) into 1
3. VI vlv - POS 2
4. 15ml of 2 into C
5. Shake tray (C mixing)
6. Contents of C (to 3ml mark) into 2

ENZYME TREATMENT

• CAUTION.

TIME LIMITS ARE CRITICAL

1. VI vlv - POS 3
2. 5ml of 3 into C
3. Shake tray FIVE SECONDS (C MIXING)
4. Allow tray to stand for TEN SECONDS
5. Contents of C (to 3ml mark) into 3
6. Allow tray to stand for TWENTY MINUTES

3

4

CELL HARVEST

1. 1ml of 3 into C
2. V1 vlv - POS 4
3. V2 vlv - OPEN

• CAUTION •

TO AVOID BACKFLOW DURING NEXT STEPS DO NOT RELEASE PRESSURE ON SP UNTIL V2 VLV IS CLOSED

4. 5ml of SP into C
5. V2 vlv - CL IMMEDIATELY, KEEPING PRESSURE ON 4 PLUNGER
6. V1 vlv - POS 3
7. Shake tray FIVE SECONDS (C mixing)
8. 2ml of C into 3

TRANSFER TO CFES SYRINGE

1. V1 vlv - POS 5
2. Contents of C (to 3ml mark) into CFES 3(4)
3. V1 vlv - POS 4
4. V2 vlv - OPEN

• CAUTION •

TO AVOID BACKFLOW DURING NEXT STEPS DO NOT RELEASE PRESSURE ON SP UNTIL V2 VLV IS CLOSED

3	4

5. 6 ml of SP into C
6. V2 VLV - CL IMMEDIATELY, KEEPING PRESSURE ON 4 PLUNGER
7. Shake tray FIVE SECONDS (C mixing)
8. V1 VLV - POS 5
9. Contents of C into CFES 3(4)
10. V1 VLV - POS 4

STERILE TRANSFER TO FSM

1. Remove CFES 3(4) from CTA
2. Remove CFES 3(4) sterile cap
3. Insert CFES 3(4) into FSM