

Standard Operation Procedure

Title: Manipulate retinal pigmented epithelium cells using optoelectronic tweezers

PURPOSE

To outline the steps required to safely handle retinal pigmented epithelium (RPE) cells in optoelectronic tweezers device ; and to describe the process for safe disposal of waste generated by working with these cells.

PRIOR TO BEGINNING WORK

All staff working with Containment Level 1 biological receive research-specific training by the Principal Investigator (PI), and complete Biosafety training in addition to all other EHS safety training.

PERSONAL PROTECTIVE EQUIPMENT REQUIREMENTS

Wear appropriate personal protective equipment (PPE) when handling paramecium and/or performing sections of this protocol:

- a. Nitrile gloves
- b. Lab coat or gown
- c. Safety glasses

MATERIALS AND EQUIPMENT

- PPE (lab coat, nitrile gloves, safety glasses);
- Yellow biowaste bin;
- Autoclave/biohazard bags;
- Pipettes and pipette tips (as needed for work);
- 1% sodium hypochlorite solution;
- Becker (for pipette tip disposal);
- Optoelectronic Tweezers (OET) instrument;
- OET devices.

Any breach of the skin (scratch, cut, wound) needs to be protected from contact with biological agents. Cover open wounds, cuts, scratches, and grazes with waterproof dressings and gloves. If you exhibit any open wounds (broken skin) in areas that cannot be covered by dressings or clothing, re-evaluate the work in process. Suggestions for mitigating the exposure in the case of broken skin that cannot be covered include, for example where the wound is on the face, work with a full-face shield; work in the BSC, or have someone else do the work.

REAGENTS

- RPE cells
- Mixture of 70% ethanol and DI water
- 1% sodium hypochlorite solution

EXPERIMENTAL PROCEDURE

1. All pipette tips used in this procedure should be discarded into a Becker containing 1% sodium hypochlorite solution and let it sit for 30 min.
2. Using pipette to load cell medium with RPE cells into the chamber of OET device
3. Label the device
4. Run experiments according to experimental protocol to be performed.
5. After using, place device in 1% sodium hypochlorite solution for 10 min;
6. Remove bleach by aspersions and discard solids (device containing microparticles and paper wicks) into yellow biowaste bin.
7. Discard non-biohazardous liquid waste in proper waste containers;
8. Discard gloves into yellow biowaste bin.
9. Wash hands with soap and water.
10. Treat liquid waste with a 1% sodium hypochlorite solution. Allow the solution to stand for at least 30 minutes.
11. Rinse solution down the drain.
12. Mop the experimental setup and working stage with 70% ethanol

WARNINGS AND PRECAUTIONS

1. Do not use the samples beyond expiration date.
2. Avoid breathing mist / vapors / spray.
3. Contaminated work clothing should not be allowed out of the workplace.
4. Wear protective gloves, protective clothing and eye protection.
5. If on skin: wash with plenty of water.
6. If skin irritation or rash occurs: Get medical attention.
7. Take off contaminated clothing and wash before reuse.

May cause allergic skin reaction. Causes eye irritation.

1. Avoid contact with skin and eyes.
2. Keep container tightly closed in a dry and well-ventilated place.
3. Wear protective gloves, protective clothing and eye protection.
4. In case of skin contact, wash off with soap and plenty of water. Consult a physician.
5. In case of eye contact, rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
6. If swallowed, rinse mouth with water. Consult a physician.