Stanford | Environmental Health & Safety
BIOSAFETY & BIOSECURITY

SORTING UNFIXED (LIVE) CELLS GUIDANCE

INTRODUCTION

Fluorescence activated cell sorting (FACS) is the process of sorting cells from a mixed population. FACS may be performed on both fixed and unfixed (live) cells. High-speed live cell sorting is considered a high-risk procedure due to production of aerosols (droplets < 5 µm), which may contain harmful biological agents. This guidance addresses requirements that protect the operator and surrounding individuals from aerosol exposure to harmful biological agents.

RISK ASSESSMENT

Prior to conducting a FACS risk assessment, users are required to determine appropriate practices and procedures. Included in this process are

1) Identification of potential biohazardous agents and evaluation of exposure to hazards.
2) Determination of appropriate biosafety level or risk group.
3) Evaluation of staff proficiency.

All human or non-human primate primary cells, cell lines, tissue or body fluids must be handled with **Universal Precautions** (https://ehs.stanford.edu/wp-content/uploads/Universal-Precautions-Flyer.pdf) utilizing biosafety level 2 practices and procedures.

DETERMINATION OF BIOSAFETY LEVEL (BSL)

For an initial biosafety level assessment use the Stanford Biosafety Manual (https://ehs.stanford.edu/manual/biosafety-manual) as a reference or contact the Stanford University Biosafety & Biosecurity office for assistance.

Work with biological agents classified at **BSL2** (or higher) as well as protocols using **non-exempt rDNA** must have approval by the Stanford University Administrative Panel on Biosafety (APB) prior to project initiation. https://ehs.stanford.edu/services/administrative-panel-biosafety-review

Flow cytometry procedures with either unfixed (live) or fixed material containing **prions** and/or **prion-like proteins** (https://ehs.stanford.edu/manual/biosafety-manual/requirements-research-prions-and-prion-proteins) must have a consultation with the Stanford University Biosafety & Biosecurity office (https://ehs.stanford.edu/about-us/biosafety-&-biosecurity) prior to any procedure due to a necessity of special procedures.

AEROSOL MANAGEMENT SYSTEM (AMS)

An AMS is designed to protect an instrument operator and surrounding individuals from potentially infectious aerosols (droplets < 5 µm).

Cell sorters used for BSL2/2+ agents should be equipped with an aerosol management or evacuation system, which is designed to evacuate aerosols from the sort chamber and the collection area.

The lack of an AMS requires an instrument to be enclosed within a Class II Biosafety Cabinet (BSC) or the operator and surrounding individuals (within 6 ft.) to wear a fit-tested respirator (N95 or equivalent) and eye protection during all aerosol/splash generating procedures.

- The AMS must be operated during sorting procedures of BSL2/2+ samples including cells that are handled under Universal Precautions.
- Aerosol containment on any new instrument must be assessed before potentially pathogenic samples are sorted.
- After initial testing, a monthly reassessment if routinely sorting BSL2/2+ is recommended.
- A reassessment is necessary after any instrument modification or HEPA filter change in the aerosol containment or management systems.

A current widely accepted non-biological method for fast efficiency testing of the AMS is performed by measuring the aerosol production with GloGerm™.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

The below PPE requirements (including lab coat, gloves and closed-toe shoes) must be followed if an AMS is not utilized or the cell sorter is not enclosed in a Biosafety Cabinet (BSC).

For BSL2/2+ eye protection and a fit tested respirator (e.g. N-95 or equivalent) must be worn while cell sorting is in progress and during all sample handling procedures that have the potential to create splashes, spills or aerosols (cap removal, loading, opening chamber, vortexing, etc.). PPE requirements apply to all personnel during a live cell sorting procedure within 6 ft. Contact the Stanford University Occupational Health Office to schedule an appointment for a N-95 fit testing. https://ehs.stanford.edu/about-us/occupational-health-center

Respirators are not required if the cell sorter is enclosed within a Biosafety Cabinet or the Aerosol Management is used and all sort and collection chambers are closed.

LOCATION

BSL2/2+:

Restrict access to the room during a sorting procedure. If possible, a cell sorter should be located where no other routine laboratory activity is performed concurrently.

BSL3:

Consult with the Stanford University Biosafety& Biosecurity group
https://ehs.stanford.edu/biosafety-specialized-information-sessions

DECONTAMINATION

After completion of work the sorter (nozzle and tubing) and work area must be decontaminated with an appropriate disinfectant. For detailed cleaning instructions follow the SOP according to the core FACS facility or the manufacture's manual.

INSTRUMENT ACCESS

Core FACS facility/department- or PI- owned instrument users must follow the owner's registration procedures, declare which biological material is being sorted, and disclose any potential biohazards. The Core FACS facility, department or PI are responsible for training of all new users.

REFERENCES

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. https://www.cdc.gov/biosafety/publications/bmbl5/

International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards. *Cytometry A. 2014 May;85(5):434-53. doi: 10.1002/cyto.a.22454. Epub 2014 Mar 13.*

Standard Safety Practices for Sorting of Unfixed Cells. *Schmid et. Al., Current Protocols in Cytometry (2007) 3.6.1-3.6.20.*How to Develop a Standard Operating Procedure for Sorting Unfixed Cells*. Methods 57(3):392-7 February 2012.*