Standard Operation Procedure (SOP)

Inactivating Genetically Modified Organisms (GMOs) with Formaldehyde Solution

The SOP refers to an inactivation of the sample material described below and under the conditions of use here:

Preconditions and precautions:

- Get properly introduced and trained to work with bacteria or viruses.
- Wear two pairs of gloves nitrile or latex gloves over each other, sleave protectors and a clean lab coat. If gloves get possibly contaminated during the procedure, change them immediately.
- Perform all step of the procedure inside a sterile work bench (Class II) until samples are covered or resuspended in the formaldehyde solution. Preparation and removal of the formaldehyde solution have to take pace in a chemical fume hood.
- Disinfect sterile work bench (Class II) with Meliseptol before and after finishing work.
- The formaldehyde solution need to be freshly prepared; in case it is older than 3 months, prepare a fresh solution according to the protocol below and document in the formaldehyde preparation log book and on the respective formaldehyde bottle!

Reagents:

- Meliseptol
- 37 % formaldehyde solution: ROTIPURAN 37 %, p.a., ACS, article no. 4979.1, Roth
- 1x PBS^{--}, pH 7.4 without calcium and magnesium (1000 mL): Dilute 100 ml 10x PBS (ROTI®Fair 10x PBS 7.4, article no. 1105.1, Roth in 1000 mL MilliQ water) in 900 ml of MilliQ water.
- 1x $DPBS^{++}$, pH 7.4 with Calcium and magnesium (Gibco)

Equipment and materials:

- Tubes or plates with lid
- Parafilm
- MilliQ water
- Vacuboy acceleration system & sterile, one-time usable plastic Pasteur pipettes
- Pipette boy & serological pipettes: 5 ml, 10m ml and 1000 ml
- Micropipette & micropipette tips: 10 µl, 100 µl, 10000 µl
- Centrifuge with aerosol tight rotor and aerosol tight rotor lids
- Sterile work bench class II
- Chemical fume hood
- 37 °C incubator

Virus or bacteria sample material:

1. Recombinant human herpesvirus 1 (**HSV-1**) of risk group 2 (produced in S2 work 92/14-2)
2. Recombinant influenza A virus (**IAV**) of risk group 2 (produced in S2 work 92/14-3)
3. Genetically modified **E. coli** Bacteria of risk group 1 such as K12-derivatives (produced or used e.g. in S1 work n° 10 of the genetic engineering annex 92/14)
4. as well as **eukaryotic cell cultures** of risk group 1, which may have been infected with the above-mentioned organisms.

Application conditions:

1. Incubation of the sample material such as **HSV-1**, **IAV** and **E. coli K12 derivatives** (see above) in a **4 % formaldehyde solution** for **30 minutes** at **room temperature** (20-25°C).
2. Incubation of cell-free **recombinant influenza A viruses** (**IAV**) in a minimum **0.015 % formaldehyde solution** for **16 hours** at **37°C**.

Preparation of formaldehyde solutions:

- For 4 % formaldehyde solution (500 mL):
  Add 54 mL 37 % formaldehyde solution to 446 mL of 1x PBS^{--}.
- For 0.015 % formaldehyde solution (500 mL):
  Add 2 mL freshly prepared 4 % formaldehyde solution (see above) to 498 mL of 1x PBS^{--}.

Document the preparation of formaldehyde solutions in the log book in lab 114.

Procedure for formaldehyde inactivation:

- Transfer closed tube or well plate containing virus or bacteria to a sterile work bench Class II
- Wash the virus or bacteria sample with 1x $DPBS^{++}$, pH7.4 and add 4 % Formaldehyde solution (not older than 3 month); in detail:
  - *For virus or bacteria grown on/in cells attached to plates,*
    - discard supernatant on cells with a sterile Pasteur pipette and the Vacuboy (take care not to scrape cells off of the well surface);
    - wash cells with 1x DPBS++, pH7.4;
    - Discard supernatant on cells with a sterile Pasteur pipette and the Vacuboy (take care not to scrape cells off of the well surface);
    - cover cells completely with 4 % Formaldehyde solution.
  - *For virus or bacteria in solution,*
    - centrifuge bacteria or viruses in solution in tubes/plates with closed lids and using an aerosol tight rotor with aerosol tight rotor lid applied;
    - resuspend virus or bacteria pellet in DPBS++, pH 7.4;
    - centrifuge bacteria or viruses in solution in tubes/plates with closed lids and using an aerosol tight rotor with aerosol tight rotor lid applied;
    - resuspend virus or bacteria pellet in 4 % Formaldehyde solution or only for cell-free IAV samples only in minimum 0.015 % formaldehyde solution.
- Close Formaldehyde container immediately after use as well as the tube or plate containing the virus or bacteria sample in formaldehyde solution.
- Clean well closed plates or tubes containing the virus or bacteria sample with Meliseptol, take out of the sterile work bench and incubate for proper inactivation with tightly closed lid:
  - *For samples in 4 % formaldehyde solution,*
    incubate for 30 min (do not incubate less, you can incubate for longer) at room temperature (20-25°C) in the chemical fume hood or
  - *For cell-free IAV samples only in minimum 0.015 % formaldehyde solution,:*
    incubate for 16 hours at 37°C in a well closed and with parafilm sealed container in at 37 °C in an incubator.
- After the proper inactivation time, transfer the samples with tightly closed lids to a chemical fume hood and discard formaldehyde solution into the formaldehyde waste container:
  - *For virus or bacteria grown on/in cells attached to plates* by accelerating the formaldehyde with a pipette boy and a serological pipette followed by adding MilliQ water or 1x PBS^{--}, pH 7.4.
  - *For virus or bacteria in solution*, by first centrifuging the tightly closed tubes/plates using an aerosol tight rotor with aerosol tight rotor lid applied and then accelerating the formaldehyde with a pipette boy and a serological pipette;
    resuspend pellet in MilliQ water or 1x PBS^{--}, pH 7.4.
- Store properly labelled sample at 4 °C for further applications.