OPERATING INSTRUCTIONS

according to §12 Genetic Engineering Safety Regulation for
the genetic engineering laboratory area of safety level 1

Institute for Biochemistry
Department of Chemistry and Biochemistry, University of Cologne
Zülpicher Str. 47, 50674 Köln

Training Laboratories

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1. Area of application

These operating instructions apply to the genetic engineering plant No. 66 in the Institute for Biochemistry, Zülpicher Str. 47, 50674 Cologne. This includes the following rooms:

160	Autoclave room
060, 070, 071	Training Laboratories
191	Cell culture laboratory
U65	Storage room
061	Training Laboratory, currenty inactive (“ruhend”)

The mentioned rooms are marked as "Genlabor S1". The break room is in room 101.

2. Responsible persons

Project leaders for the training labs (060, 061, 070, 160, 191)	In general: the Managing Director of the Institute for Biochemistry, at present: Prof. Dr. Günter Schwarz	gschwarz@uni-koeln.de
	During operation: The respective project leader: Dr. Ulrich Baumann Prof. Dr. Elmar Behrmann Prof. Dr. Karsten Niefind Prof.‘ Dr.‘ Ines Neundorf Dr. Peter Poeppel Prof. Dr. Jan Riemer Prof. Dr. Günter Schwarz	 ubaumann@uni-koeln.de elmar.behrmann@uni-koeln.de karsten.niefind@uni-koeln.de ines.neundorf@uni-koeln.de ppoeppel@uni-koeln.de jan.riemer@uni-koeln.de gschwarz@uni-koeln.de
Project leader for U65	The current Managing Director of the Institute for Biochemistry (see above), as well as: Prof. Dr. Kay Hofmann	kay.hofmann@uni-koeln.de
Biological Safety Officer:	Dr.’ Katrin Schrader	k.schrader@uni-koeln.de
Emergency/fire brigade:	-	Phone: (01) 112
Company Medical Service:	Dr.’ Susanne Knoop-Busch Michael Rysanek	Phone: 470-1915 betriebsarzt@uni-koeln.de

Responsible Hospital:	Ev. Krankenhaus Weyertal Weyertal 76	Phone: 479-1
Safety Expert:	Dr.' Annette Ahrens-Moritz	Phone: 470-2874 gentechnik@verw.uni-koeln.de
First Aiders:	See annex	

3. Genetic engineering work

Genetic engineering work of safety level S1 is carried out in the genetic engineering facility. In addition to the production, genetic engineering also includes the use, propagation, storage, destruction or disposal of genetically modified organisms as well as their in-house transport.

4. Potential hazards from GMOs

Genetically modified organisms are assigned to risk group 1. This means that if they are handled properly in accordance with these operating instructions, there is no danger to humans and the environment.

A comprehensive risk assessment is part of the records in accordance with the Genetic Engineering Recording Ordinance.

There is no additional risk for immunosuppressed persons, allergy sufferers and pregnant women.

5. Protective Measures, rules of conduct and hygienic measures

According to the basic rules of good microbiological technology and the Genetic Engineering Safety Ordinance, the following must be observed in particular:

5.1. Access regulations

a) Only those persons may work in the laboratory who have been demonstrably instructed in the necessary and project-specific safety measures at the workplace based on the operating instructions and who have the express permission of the project manager to work in the laboratory before commencing work and at least once a year may continue to work in the laboratory.

b) Visitors should only enter the laboratories in the presence of instructed personnel.

c) Cleaning and maintenance personnel may only work in the laboratories if they have been authorized by the project manager and informed at least once a year about potential danger. It is sufficient to be instructed on the type of work carried out in the laboratory and on the

essential behavioral measures. The cleaning and maintenance personnel can contact Dr. Katrin Schrader, phone. 470-7474, on site.

5.2. Personal protective equipment

a) Laboratory coats shall be worn in the laboratory.

b) Additional protective clothing (disposable gloves, multi-use gloves, goggles) must be worn if necessary. When selecting gloves, the chemical resistance specifications of the manufacturer shall be observed.

c) Disposable gloves must be disposed of after use. Contaminated disposable gloves must be autoclaved before disposal.

d) To avoid contamination, street clothing must be stored separately from protective clothing (in personal lockers).

5.3. General handling instructions

a) Before starting work, each laboratory employee must inform himself about the location and function of disinfectants, body and eye showers, first aid facilities, fire extinguishing facilities and escape and rescue routes.

b) The rooms of the genetic engineering facility must be kept tidy and clean. Only the equipment and materials actually required should be on the worktables. The supplies must be stored in rooms or cupboards provided for this purpose.

c) The writing places and computers available in the laboratories shall be kept separate from the lab benches by a splash guard. The use of writing places shall be limited to the logging of experiments. The catalogues, books, etc. available at the writing places shall be limited to the necessary extent.

d) The doors of the work rooms shall be kept closed during the performance of genetic engineering work. Windows can be opened for ventilation purposes if work is carried out during which no aerosols containing GMOs can enter the work area and if the air movement does not impair the proper functioning of safety cabinets or fume hoods.

e) Pipetting aids must be used.

f) Syringes, cannulas, blades, needles, etc. may only be used if necessary. For disposal, they must be collected in puncture-proof autoclavable containers and autoclaved. For cannulas, use cannulas with a wiping opening. Corresponding containers must be provided at the workplaces before work begins.

g) During all work, care must be taken that no avoidable aerosols occur. Aerosols may form during decanting, stirring, high-pressure pressing, inoculation, shaking, pipetting, centrifuging and ultrasonic work.

Possible measures to prevent aerosol formation:

- Use closed vessels or encapsulated working procedures.
- Before opening the containers, allow sufficient time for the aerosols to sink.
- Avoid blistering
- Observe low drop heights during decanting and pipetting
- Do not blow out pipettes, do not spray the contents of syringes/needles into the air space.
- Perform work in a safety workbench

h) The identity of the organisms used shall be regularly verified where this is necessary for the assessment of the hazard potential. The intervals shall be based on the hazard potential and the likelihood of contamination and confusion. The verification shall be carried out, for example by cultivation on selective media, restriction analysis and/or primer-specific PCR analysis.

i) The work instructions/operating instructions available on the individual devices must be observed.

j) Closed, shatterproof and labelled containers shall be used for the internal transport of genetically modified organisms. The project leader in charge shall provide these transport containers.

k) Genetically modified organisms shall be stored in suitable containers which are permanently labelled.

5.4. Supplementary instructions

Supplementary instructions and individual operating instructions for hazardous substances and devices are available separately in the genetic engineering plant.

Likewise, supplementary instructions for the containment of infections in connection with the coronavirus SARS-CoV-2 are constantly updated and all employees are informed verbally and in writing.

5.4.1. Regulations for working with adenovirus-associated vector particles (AAV)

If genetic engineering work is performed including adenovirus-associated vector particles (AAVs), two gloves must be worn on top of each other and virucidal disinfection reagents are required as listed in the hygiene and skin protection plan (see annex).

5.5. Prohibitions

a) Foodstuff, drinks, tobacco and cosmetics must not be stored in laboratories. Room 101 and personal lockers are available for storage of these items.

b) Do not eat, drink, smoke or snuff in the work rooms. The break room must not be entered with protective laboratory clothing.

c) Oral pipetting is prohibited.

d) Suction devices (e.g. water jet pumps) may only be used for liquids which may contain genetically modified organisms (GMOs) if the liquids containing the GMOs are collected in an intermediate container, thus preventing the escape of GMOs.

e) The storage of GMOs or other materials on transport routes is prohibited.

5.6. Hygiene measures

A hygiene and skin protection plan has been made, see annex. All disinfectant bottles must be marked with the use-by date (usually one year from the opening date). The transfer/refilling of the concentrates into smaller containers must be carried out under aseptic conditions. The concentrate must be stored in closed containers until usage.

5.7. Special instructions for the training laboratories

The S1-status of the training labs is activated when needed. While GMOs are being handled, the following rules apply:

a) The S1-work must be announced to the personnel timely, so that the personnel can provide the required disinfection agents and the S1-waste bins and remove them after the S1-work has been terminated.

b) All students who deal with targeted or non-targeted S1 work during this period must be instructed based on these operating instructions.

c) The respective project leader must provide suitable containers for the transport of GMOs.

d) The cold room 070 is approved as an S1 laboratory with a short stay for cell disruption of GMOs. The main work must take place in the course room.

6. Behavior in case of danger

These general rules apply:

- Keep calm, avoid hasty, ill-considered action.
- Warn endangered persons. If necessary, ask them to leave the rooms.
- Stop endangered and dangerous experiments. If necessary, turn off gas, electricity, water.

- In all emergencies, the project leader must be informed.

6.1. Leakage or spillage of biological material

If biological material is spilled, the affected area must be secured. Leaked or spilled biological material, which may contain genetically modified organisms, must be immediately inactivated. The exact decontamination measures are specified in the hygiene and skin protection plan (see annex). The project leader must be informed immediately.

6.2. Fire

For smaller fires, extinguish the fire using fire extinguishers (in the corridors). Otherwise, the valid fire protection plans must be followed. The **fire brigade** is called via phone**: (01) 112**

7. First aid

Injuries:

As far as possible, wounds should be disinfected and dressed as part of the initial treatment. Major injuries must be reported immediately to the project leader.

A doctor must be contacted in the event of exposure to or suspicion of exposure to hazardous substances.

Injuries in connection with genetic engineering work must be recorded in the injury's logbook (in the first aid cupboard, right floor on 1st floor or R071 on ground floor). These records must be kept for at least 10 years.

Inhalation or ingestion of genetically modified organisms:

The project manager must be informed immediately, and medical advice obtained as to whether and how treatment is necessary. The project leader and the attending physician must be informed which organisms have been ingested and in what quantity.

8. Proper disposal

- Solid and liquid waste containing GMOs must be inactivated before disposal.
- Inactivation is carried out by autoclaving (autoclave room 160). Closable containers and autoclave bags must be kept wide open during autoclaving.
- Other liquid wastes are collected separately by the staff and autoclaved in the original containers.
- Solid waste is collected until inactivation in appropriate autoclavable bags in a solid, shatter-proof container in the laboratories. The inactivation shall be performed in approved

autoclaves (e.g. in Facility 367, room 255: in Systec DX-90 at 121°C for 20 min, or in VX-190 at 134°C for 20 min). It must be ensured that these wastes do not contain any hazardous substances!

- The functionality of the autoclaves is checked every six months and after maintenance/repair using bioindicator tests. These bioindicators are placed in the autoclave according to the manufacturer's instructions and then cultivated. After maintenance/repair, the bioindicator test is carried out before the autoclave is put back into operation.

9. References to general rules and regulations

Laws/Regulations

- Genetic engineering law
    - Genetic engineering safety ordinance incl. Annexes 1-6
    - Genetic engineering recording ordinance
- Hazardous Substances Ordinance with the following technical rules for hazardous substances
    - TRGS 526 Laboratories
    - TRGS 555 Operating instructions
- Ordinance on Industrial Safety and Health with the Technical Rules for Industrial Safety and Health
- Radiation Protection Ordinance
- Ordinance on Biological Substances
- Maternity Protection Act
- Working Conditions Act
- Animal Welfare Act

Publications

- List of risk-rated donor and recipient organisms for genetic engineering work (website of the Federal Office of Consumer Protection and Food Safety: www. bvl.bund.de)
- List of disinfectants and disinfection methods tested and approved by the Robert Koch Institute (www.rki.de)
- Disinfectant list of the Verbund für Angewandte Hygiene e.V. (Association for Applied Hygiene)

Employer's liability insurance association regulations

- Safe working in laboratories (DGUV Information 213-850)
- Principles of prevention (DGUV regulation 1)
- Company physicians (DGUV regulation 8)
- Occupational health care (DGUV regulation 7)
- Operation of work equipment (DGUV rule 100-500)
- Dangerous substances at universities (DGUV Information 213-044)
- Information from the Employer's Liability Insurance Association "Safe Biotechnology“
    - Fachbegriffe (BGI 628)
    - Laboratorien (BGI 629)
    - Betrieb (BGI 630)
    - Viren (BGI 631)
    - Parasiten (BGI 632)
    - Prokaryonten (BGI 633)

- Pilze (BGI 634)
- Gentechnisch veränderte Organismen (BGI 635)
- Zellkulturen (BGI 636)

DIN Guidelines

- DIN EN 14056 Laboratory equipment
- DIN 12469 Performance criteria for safety cabinets
- DIN 58951 Steam sterilizers for laboratory sterilization goods
- DIN EN 12884 Performance criteria for centrifuges
- VDI 6300 Guideline for the safe operation of genetic engineering plants

10. References to special regulations

Duty to notify:

The project leader must be informed of any incident that does not correspond to the expected course of the genetic engineering work.

Instruction:

Before the start of work and at regular intervals (at least once a year), the employees must be instructed based on the operating instructions for the workplace. The content and time of the instruction must be recorded in writing and confirmed by the instructed persons by signature.

Duty to keep records:

Only genetic engineering work of safety level 1 is permitted in the facility. These operations must be recorded using Form Z. Since the information on the donor and recipient organism, the vector and the transferred gene is an essential part of the risk assessment of genetic engineering work, this information must be included in the records. The records must be kept for at least 10 years after completion of the relevant genetic work.

Penalties and fines:

Violation of the provisions of genetic engineering law may result in fines of up to € 50,000 and punitive measures of up to 5 years imprisonment. Furthermore, claims for damages of up to € 85,000,000 may be incurred due to liability regulations under genetic engineering law.

11. Annexes

- List of first aiders in the Institute for Biochemistry
- hygiene and skin protection plan
- supplementary instructions for equipment and hazardous substances