Stand: Datum

**Safety Instructions**

according § 17 part 2 GenTSV

**for Research Work in Genetic-Engineering**

**Laboratories**

**Safety Level 2**

Content

[1. Scope 2](#_Toc484698292)

[2. First Aid, dangerous situations 2](#_Toc484698293)

[2.1. Injuries 2](#_Toc484698294)

[2.2. Fire 2](#_Toc484698295)

[2.3. Leakage, Spillage of biological material 2](#_Toc484698296)

[3. Key people 3](#_Toc484698297)

[4. Genetic engineering work 3](#_Toc484698298)

[4.1. Risk assessment and work records 3](#_Toc484698299)

[4.2. Storage and Transport of GMOs 3](#_Toc484698300)

[4.3. Disposal of GMOs an contaminated waste 4](#_Toc484698301)

[5. Access, briefings and medical examinations 4](#_Toc484698302)

[6. General rules, safety instructions 4](#_Toc484698303)

[6.1. Personal protective equipment 5](#_Toc484698304)

[6.2. Rules 5](#_Toc484698305)

[6.3. Work with transgenic animals 5](#_Toc484698306)

[6.4. Supplementary instructions 5](#_Toc484698307)

[6.5. Prohibited activities 6](#_Toc484698308)

[7. Hygiene 6](#_Toc484698309)

## Scope

|  |  |
| --- | --- |
| Reference number: | 40611/xxx/xxx |
| Rooms | Room number and function (e.g. Laboratories: 01 D3 234, 235, 236; autoclave room: 01 D3 237 etc.) |

The rooms above are marked with Safety Level 2 and the Biohazard symbol.

## First Aid, dangerous situations

|  |  |  |
| --- | --- | --- |
|  | First-aid kit | Room xx |
| Fire, Rescue, Emergency | 112 |
| Central Failure and Alarms Management | 1171 |
| Emergency poison center | 22032 |

* Keep calm and avoid hasty actions.
* Warn people in danger; ask them to leave the rooms if they are not already doing so.
* Stop experiments that are at risk or are dangerous; switch off the gas, electricity and water.

(The emergency shutdown switch for… is located…; it may be necessary for cooling water not to be turned off).

* The project manager must be notified immediately of any injuries.
* Injuries must be recorded in the first-aid log (“Verbandbuch”) in room number.
* Follow instructions on the emergency notice “Alarmblatt”.

### Injuries

1. Disinfect contaminated skin: substance? concentration? Exposal time?
2. Wash eyes and mucous membranes thoroughly (at least for 5 minutes) with copious amounts of running water.
3. If symptoms persist in spite of immediate measures, the injured person must come straight to the Day Clinic or the Night Clinic (TN) UBFT, Level 01, Lift C 1. The doctors should be informed of the danger of infection with biological material.

### Fire

Small fires must be put out using the extinguishers in rooms *room numbers*. For all other fires, please follow the instructions in the applicable fire protection plans (”Brandschutzpläne”).

### Leakage, Spillage of biological material

If biological materials leak or are spilt, the area must be secured and the materials and surfaces affected must be treated in order to inactivate the genetically modified organisms.

The following decontamination measures must be taken:

- Surfaces: Put protective gloves on. Carefully pick up the leaked or spilt material using autoclavable material (e.g. paper towels), being particularly cautious if there is broken glass, and then autoclave it. Afterwards, disinfect the contaminated area according to the disinfection plan.

- Appliances: See “Surfaces”.

 Where alcohol disinfectants are used, explosion protection requirements must be complied with, particularly on electrically operated appliances and systems used in conjunction with naked flames or hot surfaces (see Section 7d).

- Clothing: Remove protective/street clothing and treat it as described in the disinfection plan. Afterwards, wash the garments.

- Skin: Disinfect contaminated areas of skin as described in the disinfection plan; rinse with plenty of water after the contact time has elapsed.

- Eyes: Rinse eyes well (approximately 10 minutes) using the eyewash unit in room room number. To prevent eyelids closing reflexively, use thumbs and index fingers to hold eyes open. In case of injury or chemical burns, consult the closest eye specialist immediately.

- Mucous membranes: Rinse contaminated mucous membranes with plenty of water. If necessary, consult the closest doctor.

## Key people

|  |  |  |
| --- | --- | --- |
| Project manager | Name | Telefon |
| Biosafety officer (BBS) | Name | Telefon |
| Security officer (“Sicherheitsbeauftragter”) | Name | Telefon |
| Radiation protection officer (“Strahlenschutzbeauftragter”) | Name | Telefon |
| Laser protection officer (“Laserschutzbeauftragter”) | Name | Telefon |

## Genetic engineering work

The genetic engineering facility is used for safety level 2 genetic engineering activities concerning the following topics:

*Please list the used organisms with the respective risk group. Please also specify the additional S2 works including the official reference number (“Aktenzeichen”) and the project managers responsible for them*.

In addition to growing genetically modified organisms (GMOs), the genetic engineering activities include the use, propagation, storage, destruction, disposal and in-house (within the facility) transport of GMOs.

### Risk assessment and work records

The genetically modified organisms are classified no more than risk group 2. Prior to the planned genetic engineering activities, a risk assessment must be carried out for them, indicating the safety level of the genetic engineering activities (S1 or S2). The risk assessment forms part of the records required by the Regulations on Genetic Engineering Records (“Gentechnik-Aufzeichnungsverordnung”) and has to be stored at least for 30 years.

### Storage and Transport of GMOs

* + 1. Genetically modified organisms must be stored in suitable containers. To ensure there are no mix-ups, the containers must be marked using permanent ink/labels.

Bacteria, etc. to be stored at -xx°C, are stored in the freezer in room …

Cells, etc. to be stored at -xx°C, are stored in the freezer in room …

Cells, etc. to be stored in liquid nitrogen, are stored in room …

* + 1. Freezers for GMO storage must be labeled with S2 and the biohazard symbol. Freezers outside the S2 area have to be officially registered and kept locked to prevent unauthorized access!
		2. **Transport of GMOs**: Sealed, non-breakable containers labeled with S2 and the biohazard symbol are used for transport of GMOs within the facility.

### Disposal of GMOs an contaminated waste

1. Solid and liquid waste containing genetically modified organisms must be inactivated prior to disposal. This is done by autoclaving it for 20 minutes at a temperature of 121°C. The following autoclaves can be used:

|  |  |  |
| --- | --- | --- |
| Type | Floor | Room number |
|  |  |  |

1. Autoclave Features in S2:

Mandatory features: exhaust air filtration, condensate inactivation and vacuum system. The filter is changed every xx runs or every xx month.

Every **6 month** the sterilization process is confirmed (and documented!) with Bioindicators, e.g. Sterikon plus (Merck).

1. Transgenic animals:

Animal cadavers infected with pathogenic (!) microorganisms (e.g. bacteria, viruses) must be autoclaved!

All cadavers are collected at -20°C until they are picked up. All contaminated solid waste has to be autoclaved.

1. Radioactive GMO waste:

Autoclaving radioactive waste is prohibited! The waste must be inactivated by chemical means; this procedure requires official permission from the authorities! Chemical waste inactivation is permitted on the following criteria: Give information about GMO, substance, concentration and min. exposure time

## Access, briefings and medical examinations

* + 1. **Entry** only for authorized persons. Authorization can only be granted by the project manager. External personnel may only enter with permission from the project manager and after specific instructions have been given (instructions must be signed!). This also applies to the cleaning staff. **Changes in state of health** (e.g. weakened immune system on account of medication, pregnancy, open wounds, eczema of the skin of the hands etc.) must be reported to the head of the project. Such changes should lead to prevention of such persons from working with genetically modified organisms of the risk group 2 or to special protective measures.
		2. All authorized persons will be instructed according to this set of instructions by the head of the project before the project has begun, if changes affecting safety occur, immediately, and otherwise once a year verbally and with specific reference to the place of work. These **instructions** are obligatory for authorization and the authorized person must sign that he has received and understood them.
		3. **Occupational medical examinations** are performed in accordance with the provisions of the Regulations on Preventive Occupational Healthcare (see ArbMedVV, Annex, Part II). These examinations are compulsory for all employees who are to work at these workplaces. Optional occupational health examinations are provided for the majority of the organisms on a voluntary basis.

## General rules, safety instructions

In accordance with good microbiological practice and the provisions set out in the Genetic Engineering Safety Regulations (“Gentechnik-Sicherheitsverordnung, GenTSV”), the points below must be adhered to in particular:

### Personal protective equipment

1. Lab coats and other safety precautionary clothing are to be worn in S2 rooms. Safety precautionary clothes are to be removed before exiting the S2 rooms and stored separately from normal street clothing.
2. Disposable gloves must be disposed of after use. Contaminated disposable gloves must be autoclaved and then disposed of as solid waste.

### Rules

* + 1. Keep doors and windows closed when work is in progress.
		2. In S2 laboratories only brief written documentation, but no **writing desks** are allowed.
		3. Before beginning their duties, all laboratory employees must make sure they know where the disinfectants, safety showers, eyewash units, first aid equipment and fire extinguishing devices are and how they work as well as determining where the evacuation and emergency exit routes are.
		4. The rooms in the genetic engineering facility must be kept clean and tidy. Only the appliances and materials actually required must be placed on the work tables.
		5. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
		6. Sharp or pointed equipment (e.g., hypodermic needles, syringes, and scalpels) must not be used unless necessary.
		7. **Formation of aerosols** must be avoided. Aerosols are likely to form for example in the following processes: decanting, stirring, high-pressure compression, inoculating, shaking, pipetting, centrifuging and working with ultrasound. **All activities involving risk group 2 organisms must be carried out in a class 2 biological safety cabinet!**

Guidelines to prevent aerosol formation:

* Use closed containers or enclosed work processes
* Before opening containers, give the aerosols sufficient time to settle
* Avoid formation of bubbles
* Minimize the height of fall when decanting and pipetting
* Do not blow out pipettes or spray the contents of syringes/hypodermic needles into the ambient air.
	+ 1. **Centrifugation of S2-organisms**: Always use sealed safety cups, safety buckets, or sealed rotors with O-ring as secondary containment if available. The last especially when the centrifuge is too big to take it under a biological safety cabinet in case of a broken tube! The centrifuges cups may only be opened under the class II biological safety cabinet. **Emergency procedures**: If the centrifuge indicates a problem in the vacuum system, this could theoretically be a defective, leaking rotor. Cool the rotor chamber down to 0°C to disperse aerosols, open the rotor (or centrifuge) only under a biological safety cabinet and clean the rotor chamber immediately with disinfectant.
		2. The identity of the organisms used must be verified on a regular basis if necessary in order to assess risk potential
		3. The work instructions (“Betriebsanweisungen”) attached to the centrifuges, autoclaves, biological safety cabinets, microwave ovens, *etc.*, which include safety information, must be complied with.

### Work with transgenic animals

Please specify rules regarding transport, Identification, safety instructions, protective equipment (technical, personal, organizational).

### Supplementary instructions

* + 1. Handling **cryogenic liquid nitrogen** (LN): there is a risk of a dangerous drop in the oxygen content of the air in the room due to nitrogen being added to it. Precautions when handling LN can be found in the work instruction (“Betriebsanweisung”) entitled “Storage and handling of liquid nitrogen”.
		2. Handling potentially **oncogenic nucleic acids** (see Recommendation by the Central Committee on Biological Safety (ZKBS), ref. no. 6790-10-01 and 6790-10-36):
* Disposable gloves must be worn when working with such nucleic acids.
* Use of sharp, pointed or fragile laboratory utensils should be avoided.
* Laboratory workstations and appliances that come into contact with such nucleic acids must be cleaned thoroughly once the activity has been completed.
* Laboratory waste containing such nucleic acids must be denatured by means of autoclaving or chemical treatment.
* Individuals with significant skin lesions (open eczema sores, wounds or infections) or pronounced verrucosis (multiple warts) must not work with these types of nucleic acid.
	+ 1. Handling of **adenoviral vectors, which overexpress potential oncogenes** (ZKBS 6790-10-83):
* Gloves are either to be disinfected or changed regularly
* The use of a respirator mask with FFP3-filter is obligatory
* Containers and devices are to be disinfected from the outside before removing them from the safety cabinet
* Cell culture bottles containing adenoviral vectors, are not to be ventilated before entering the CO2-incubator, to avoid leaking of culture medium

Alternatively, the work can be carried out under the following safety measures:

* Usage of a biological safety cabinet class III to avoid aerosols.
* The vectors must be kept in tightly closed, non-breakable containers, that are disinfected from the outside. Opening, closing an d disinfection of the container is to be performed in the class III safety cabinet.
	+ 1. When working with **lentiviral vectors** for transduction of nucleic acid fragments with **oncogenic potential**, the use of mouth and nose protectors to avoid smear infection is obligatory. This is particularly important when working with particles with increased stability or increased effect spectrum for human epithelial cells upon pseudotyping or which can’t be recognized by the human complement system due to changes in the glycosylation pattern. (ZKBS 6790-10-41).
		2. Downgrading of transduced cells:

Before cells are transferred to an S1 area after viral transduction, the following conditions must be ensured:

Viral particles should eliminated in the supernatant by xx repeated washing steps or passages after transduction. If transduced cultures are spent at an earlier stage in an S1-area, the absence of particles must be verified by test series.

### Prohibited activities

* + 1. Do not eat, drink, smoke, chew or inhale tobacco products, apply cosmetics, or store food or stimulants in the containment facility.
		2. Mouth pipetting is prohibited.
		3. Storage of gas bottles is not permitted. Where it is absolutely necessary to use compressed gas cylinders, they must be positioned, handled and, in particular, prevented from being knocked over using the measures set out in TRGS 526 (Technical Rules for Hazardous Substances; Laboratories), Section 5.2.11.
		4. Other prohibited activities:

## Hygiene

1. A cleaning and **disinfection plan** has to be provided containing effective products and information`s regarding the correct handling.
2. Upon completion of an activity and prior to leaving the work area, individuals must, if necessary, disinfect their hands, wash them thoroughly and moisturize them (see the **skin protection plan**).
3. Disinfectants for surfaces must be applied using wash bottles and then rubbed on the damp surface by mechanical action (wiping technique). Spray bottles may only be used in difficult-to-reach places because the active ingredients can easily be breathed in as gases or aerosols, which can result in toxicological effects and allergies when used regularly.
4. Where alcohol disinfectants are used, explosion protection requirements must be complied with, particularly on electrically operated appliances and systems that are used in conjunction with naked flames or have hot surfaces. Hot surfaces – including surfaces inside appliances – must cool down before being disinfected. The room must be sufficiently ventilated when alcohol disinfectants are being applied. A maximum of 50 ml of alcohol disinfectant working solution may be used per square meter of surface to be treated.

|  |  |  |
| --- | --- | --- |
|  | **date** | **signature** |
| **Project manager** |  |  |
| **BBS** |  |  |