# Operating instructions for biological substances

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| **Organisation:** | Heidelberg University  Institute of Neurobiology | **Rooms:** | INF 366, rooms 123-127, 145 | |  |
| **Research Groups:** | Bading, Cicciolini, Mauceri, Oliveira | **Activities:** | Transformation, plating, and outgrowth of E. coli on agarose plates and in liquid culture; purification of plasmid DNA from liquid cultures | | |
| **Responsible Person:** | Prof. Hilmar Bading | **Created by:** | Dr. Anna M. H. Hertle | **Date:** | 21.11.2023 |
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**Description**

# *E. coli*

*E. coli* strains such as K12, DH5α, NEB5⍺, NEB Stable cells, etc. are used for the development, propagation and preparation of recombinant DNA. Attenuated strains of *E. coli* typically used for molecular biology are considered Risk Group 1 (RG1) agents, which are not associated with disease in healthy adult humans and require Biosafety Level 1 containment. Please note that genetic modifications imparting virulence factors1 or modifying the regulation of gene expression may increase the level of invasiveness and virulence, requiring elevated containment and safety practices commensurate with the level of risk. Therefore, this risk assessment DOES NOTpertain to *clinical isolates* which are considered *Risk Group 2* organisms capable of exhibiting greater virulence and requiring containment at Biosafety Level 2.

## Hazards for humans and the environment

**Risk Group 1 laboratory strains of** E. coli **have a low, but not non-existent, virulence. Although organisms classified as Risk Group 1 are not known to consistently cause disease in healthy adults, the following individuals may be at increased risk of infection: the young, elderly, immunocompromised individuals or individuals taking large quantities of antacids or stomach acid reducing medications.**Possible routes of exposure include inhalation of aerosols, ingestion, and contact with mucous membranes (including eyes). **Inflammation may occur if autoinoculation results from injection or contamination of broken skin. Ingestion of E. coli may cause diarrhea with or without abdominal cramps. Skin infections may be inflamed. Conjuctivitus may occur after inoculation of the eye.**

## Safety measures and codes of conduct



* Work may only be performed in an S1 area with adequate ventilation. Standard S1 precautions must be adhered to.
* Wear a lab coat, eye goggles, and gloves.
* Disinfect and wash hands prior to leaving the S1 lab.
* Handle liquids carefully to minimize the creation of splashes and aerosols. Centrifugation should be performed using sealed tubes. Vessels used for orbital shaking must be lidded.

## Decontamination

* Regular cleaning of work surfaces and pipettes may be carried out using 70% ethanol.
* Spills should be disinfected by soaking the affected area with Baccilol AF for a minimum of 1 minute.
* Decontaminate reusable glass- and plasticware by soaking in 1.5% Lysoformin for a minimum of one hour or in 1.5% Sekusept Plus for four hours.
* Decontaminate lab coats by autoclaving.

## Conduct in case of danger

In case of accidents or spills:

* Notify workers in the area.
* Leave the area for a minimum of 15 minutes to allow aerosols to settle. Replace contaminated PPE. Initiate spill response procedure:
* Cover the spill with absorbent material. Start at the edges and work towards the center.
* Carefully pour Baccilol AF disinfectant over the absorbed spill, again starting at the edges and saturating the area with disinfectant.
* Allow a sufficient contact period (minimum 10 minutes) to inactivate the material in the spill.
* Use paper towels to wipe up the spill. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves.
* Discard absorbant material in autoclave waste.
* Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area and allow to disinfect for the required period of time, then wipe up the area with towels.
* Discard all cleanup materials in autoclave waste and send any contaminated PPE for autoclaving. Close and secure the bags.
* Disinfect the bags prior to disposal by autoclaving.

Report all incidents to supervisor.

## First aid

* Contact with clothing: remove immediately and wash the affected skin with antiseptic soap and a high volume of water for up to 15 minutes.
* In case of skin contact: wash the affected area with antiseptic soap and a high volume of water for at least 15 minutes. Consult a physician.
* In case of ingestion: rinse mouth with water, but do not swallow, then consult a physician immediately.
* In case of inhalation: move to the fresh air, then consult a physician immediately.
* In case of eye exposure: flush with water for at least 15 minutes. Consult a physician.
* Report all incidents to supervisor and make a record in the first-aid book.

## Proper waste disposal

* Dispose of all solid waste, including gloves that were used to handle E. coli and its containers, in an autoclave bag. Close and secure the bag, and disinfect by autoclaving (> 1 hour at 121°C).
* Dispose of all liquid waste in an autoclave jug. Close the jug and decontaminate the outside with Baccilol AF disinfectant. Disinfect the contaminated liquid by autoclaving (> 1 hour at 121°C).

I hereby confirm that I have read the operating procedures for working with E. coli, and agree to follow these procedures.

Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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