

## Production of Monoclonal Antibodies



### General Aim

The production of one type of antibody derived from a single cloned B cell.

### Method

Hybridoma technology.

### Learning Objectives (ILOs)

- Practice sterile cell culture techniques.
- Evaluate the need to passage cells i.e. assess confluency.
- Handle mice for immunization, including IV tail injection and intraperitoneal injection.
- Practice serial dilution of serum.
- Prepare single cell suspension out of the spleen of mice.
- Perform the fusion technique using PEG.
- Treat Hybridomas with HAT.
- Prepare hybridomas for flow activated cell sorting FACS.
- Screen hybridoma colonies for specific antibody production using ELISA.
- Freezing hybridomas.

### Theoretical Background/Context

The production of a monoclonal antibody requires several months. The general procedure includes the following steps:

- Immunization of mice with the antigen of interest.
- Screening of mice antibody titer using ELISA. This step can tell if animals' immune systems responded to the antigens injected. It also indicates which animal has the highest response.
- Thawing and preparation of myeloma cells
- Preparation of single cell suspension from mice spleens.

### Theoretical Background/Context (Cont')

- Fusion of antibody-producing B cells from spleens and myeloma cells.
- Selective growth of the hybridoma in HAT.
- Screening and sorting the hybridomas for producing the desired monoclonal antibody using FACS.
- Cloning of selected hybridomas.
- Screening of hybridomas for antibody production using ELISA.
- Freezing of the hybridoma.

### Principle of Work

The first step is Immunization, where a mice is injected with the antigen, to which an antibody is to be generated. The rat's immune system responds to the antigen by producing B cells. Each B cell produces a single type of antibody.

B cells are isolated from the mouse's spleen and fused with immortal myeloma cells producing 'hybridoma cells'. This fusion is done with the help of polyethylene glycol (PEG).

Hybridomas should be able to produce the desired antibody. They are sorted using flow cytometry (FACS). Thus; hybridomas can be cloned into a 96 well plate in a 1cell/well ratio. When colonies become confluent, they can be screened for antibody production using ELISA.