

Identification of xenografted human stem cell-derived hepatocytes in frozen mouse liver slices using recombinant monoclonal rabbit antibodies

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
This method makes use of	Other (e.g. bacteria): Recombinant antibodies that are produced in vitro

DESCRIPTION

Method keywords

immunohistochemical staining
recombinant antibodies
frozen sections

Scientific area keywords

stem cells
liver disease

Method description

In this method, we use rabbit monoclonal antibodies that are produced *in vitro* to identify xenografted human stem cell-derived hepatocytes in frozen murine liver slices. These recombinant antibodies were produced *in vitro* by cloning antibody genes for immune-specific heavy and light antibody chains into high-yield expression vectors. These vectors are then introduced into expression hosts (eg bacteria, yeast, or mammalian) to generate the recombinant monoclonal antibodies.

Lab equipment

- Fluorescence microscope ;
- PAP pen for immunostaining.

Method status

History of use

PROS, CONS & FUTURE POTENTIAL

Advantages

Recombinant antibodies are produced *in vitro* by several commercial companies and offer several advantages over traditional monoclonal and polyclonal antibodies:

1) ~~Improved consistency and reproducibility~~: Recombinant antibodies are developed from a unique set of genes and therefore antibody production can be controlled and is reliable. Several problems with hybridoma production can as such be avoided, such as gene loss, gene mutations, and cell-line drift. Recombinant produced antibodies, therefore, suffer from very little batch-to-batch variability.

2) ~~Improved sensitivity and specificity~~: With recombinant technology, it is easier to improve both antibody specificity and sensitivity through antibody engineering. The selection process for the desired clone occurs at both the hybridoma and recombinant cloning stages, allowing to select the most favorable antibody qualities before production.

3) ~~Ease of scalability~~: With the antibody genes isolated, antibody expression can be carried out at any scale and in a shorter timeframe than traditional monoclonal technology. This means that tailored antibodies can be generated in weeks rather than months.

4) ~~Animal-free high-throughput production~~: Once the antibody-producing gene is

isolated, animal-free *in vitro* production can be implemented. For antibodies generated using phage display technology, even the gene of the antibody can be isolated with an animal-free procedure.

Using these types of antibodies we were able to specifically label human stem cell-derived hepatocytes in mouse livers after transplantation and discriminate them from the endogenous mouse hepatocytes.

Challenges

There are several challenges related to recombinant antibodies:

- 1) Most recombinant antibodies are still first generated using animals in order to isolate the antibody-producing gene and only can be subsequently produced recombinantly once this gene is isolated.
- 2) For some difficult targets, recombinant libraries will not be adequate to provide a valid recombinant antibody.

Future & Other applications

Recombinant antibodies can be used in a plethora of applications including ELISA, flow cytometry and even clinical applications.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

[IHC on frozen sections using recombinant antibodies.pdf](#)

PARTNERS AND COLLABORATIONS

Organisation

Name of the organisation Vrije Universiteit Brussel

Department Pharmaceutical Sciences and Pharmacology

Country Belgium

Geographical Area Brussels Region

Coordinated by



Financed by

