One clone, one antibody type

Monoclonal antibodies are identical antibodies produced by a single cell clone. They share the same binding-site structure and recognise the same epitope on an antigen.

1. WHY MAKE A HYBRID CELL?

An antibody-producing B-lymphocyte supplies the desired antibody specificity, but has a limited lifespan in culture. A suitable myeloma cell supplies the capacity for sustained division. Fusion combines these useful properties.

2. THE HYBRIDOMA PRINCIPLE

B-lymphocyte

From an immunised animal. Can contribute production of an antibody against the target.

Myeloma cell

A tumour-derived cell line. Divides repeatedly; does not supply the required antibody.

Cell fusion

Hybridoma

A hybrid cell that can divide repeatedly and secrete antibody. The useful producers must still be identified.

3. FUSION IS ONLY THE START

A fusion mixture contains different cells and antibody specificities. It is not yet a purified source of one desired monoclonal antibody.

Hybridoma principle and monoclonality: OpenStax, Microbiology 20.1.
Parent-cell properties and fusion: National Research Council, chapter 1.

Scope: Cambridge Biology 9700 syllabus, section 11.2, outcome 2, p. 31.

From antigen to antibody supply

The classical hybridoma method uses immunisation, cell fusion, selection, screening and cloning. Each stage solves a different problem.

1. IMMUNISE

Expose an animal, commonly a mouse, to the target antigen. This stimulates an antibody response.

2. COLLECT AND FUSE

Collect antibody-producing B cells, typically from the spleen, and fuse them with suitable myeloma cells.

3. SELECT HYBRIDOMAS

Use selective culture conditions so hybridomas can grow. Unfused myeloma cells are eliminated; unfused B cells do not survive indefinitely.

4. SCREEN FOR THE TARGET

Test the antibodies secreted by surviving cultures. Keep a culture whose antibody binds the desired target.

5. CLONE A SINGLE CELL

Isolate one suitable hybridoma and grow its descendants. Recheck that the clone secretes the required antibody.

6. EXPAND AND HARVEST

Grow the selected clone in culture. Collect and purify the secreted antibodies from the culture fluid.

Immunisation, fusion and selection: National Research Council, chapter 1.
Screening, recloning and stable cultures: JoVE, hybridoma technology (2017).

Scope: Cambridge Biology 9700 syllabus, section 11.2, outcome 2, p. 31.

Why screening and cloning matter

A successful fusion is not enough: the cells must secrete a useful antibody, and the production culture must come from a single selected clone.

1. SELECTION IS NOT SCREENING

Selection favours survival and growth of hybridomas. Screening tests what antibody they secrete. A surviving hybridoma may make an antibody with the wrong specificity, so survival alone cannot identify the desired producer.

2. A POSITIVE CULTURE IS NOT NECESSARILY ONE CLONE

A culture can initially contain more than one hybridoma. Single-cell cloning separates lineages. The chosen lineage is expanded and checked again for stable production of the required antibody.

3. MONOCLONAL VERSUS POLYCLONAL

Monoclonal

One clone; one antibody type directed at one epitope.

Polyclonal

Several B-cell clones; a mixture recognising different epitopes.

An antigen can have several epitopes. Monoclonal does not mean every possible antigen-binding site on the target is recognised.

4. KEEP CELLS AND PRODUCTS SEPARATE

Hybridoma cells divide by mitosis; antibody proteins do not divide. The cells secrete antibodies, which are then collected. A maintained, productive clone can provide repeated batches with the same binding specificity.

Screening and monoclonal cultures: JoVE, Generation of Murine Monoclonal Antibodies.
Monoclonal versus polyclonal production: OpenStax, Microbiology 20.1.

Scope: Cambridge Biology 9700 syllabus, section 11.2, outcome 2, p. 31.