

Getting Started with EZ-Flask for Hybridomas

Introduction

EZ Flask (KDW0010) is a unique, 1 Liter, single compartment, **hybridoma culture flask** with a 200cm² basal gas exchange membrane. After inoculation with hybridomas, or other suspension cells, the cells will gravitate to the base and settle above the membrane. The cells remain at the gas interface and are continuously oxygenated as they proliferate to very high numbers. This means 1 Liter of high titre monoclonal antibody can be obtained without the need for passaging or manipulations that require special care.

Simple technical points to remember

To initiate the culture, enough cells need to be introduced to create a growth-boosting environment; 50 to 100 million cells in the case of Procedure 1, below. Procedure 2 only requires 10 million cells but in this case, or when using serum free media, a lower starting volume of media should be used, working up to 1 Liter in 250ml steps.

Some hybridomas only produce antibody during exponential growth in EZ-Flask whereas others can continue to secrete a lot of antibody in the plateau phase or beyond. In the latter case waiting until a cell viability is down to 10% prior to harvesting will optimize productivity.

Example procedures for EZ-Flask

Procedure 1 – simplest approach : “fill and forget”

Grow 50 to 100 million cells in T-Flask.

DAY 0 : inoculate 1 L of media (e.g. DMEM/10%FBS) in EZ Flask.

Leave in incubator undisturbed.

DAY 20 to 30 : Harvest 1 Litre of monoclonal antibody.

Procedure 2 – fewer cells to inoculate or serum-free media

Grow 10 to 50 million cells in T-flask.

DAY 0 inoculate cells into **250ml** of media (e.g. DMEM/10%FBS).

DAY 2 or 3 add **250ml** to double the volume to 500ml.

DAY 7 add 500ml to make the total volume up to 1 Litre.

DAY 14 – 16 harvest 1 Litre of monoclonal antibody.

When using **serum-free media** we recommend to inoculate only 250ml, then add 250ml every following week up to 1 Litre, and then harvest during the week after the final addition.

By starting with a low volume and steadily increasing volume, the cells are better able to condition the media which helps establish a robust culture .

Optimizing with the G-Rex 6-Well Plate

The G-Rex 6-Well Plate (KDW0026) employs the same technology and provides a convenient way to experiment with different conditions prior to scaling-up with the EZ-Flask. G-Rex 6 wells each have 10cm² gas permeable membrane with 40 mL media capacity.