QED Fuse-It® Hybridoma Development Kit provides the key reagents needed to create mouse and rat hybridoma cell lines. All of these reagents are sterile and pre-qualified for supporting maximum growth of hybridoma cell lines. Each kit contains:

1. 2 x 2 g Polyethylene Glycol (PEG)
2. 50X Hypoxanthine-Aminopterin-Thymidine (HAT)
3. 50X Hypoxanthine-Thymidine (HT) (see Note)
4. 50X 8-Azaguanine
5. 10 ml BriClone Hybridoma Cloning Medium (QED catalog no. BRI10000)

Required but not provided:
Cell culture medium such as DMEM or IMEM, fetal bovine serum (FBS), and L-glutamine. Addition of penicillin-streptomycin (10,000 units/ml penicillin/10,000 ug/ml streptomycin)at 1% is optional.

Preparation of Myeloma Cells

Myeloma cell lines for fusion with mouse and rat spleen cells (such as mouse Sp2/0 or P3X63-Ag8.653, rat YB2/0) are available from the American Type Culture Collection (Tel. 301-881-2600). Prepare medium for myeloma cells as follows: reconstitute lyophilized 8-azaguanine with 10 ml culture medium (medium + 10% FBS + 2% L-glutamine + 1% penicillin-streptomycin (optional)). This will be a 50X solution of 8-azaguanine. Dilute this solution 1:50 in the same culture medium as above for use. Myeloma cells are cultured in this medium containing 8-azaguanine for at least two (2) weeks prior to fusion.

Fusion Procedure

QED provides the following procedure with the assumption that the user has had experience producing hybridoma cell lines. For more details on this process, see *Current Protocols In Immunology* (Volume 1, Chapter 2, John Wiley & Sons, New York).

Pre-warm cell culture media in a 37°C water bath: one bottle of culture medium + 10% fetal bovine serum (FBS) + 2% L-glutamine + 5% BriClone Hybridoma Cloning Medium + 1% penicillin-streptomycin (optional) and one bottle of serum-free culture medium + 2% L-glutamine. The serum-free culture medium is the wash medium, and the serum-containing medium is the complete medium. All media should be sterilized by filtration through a 0.22 μm filter before use.

I. Preparation of Spleen Cells

A. Isolate spleen cells by asceptically teasing apart the spleen in a dish of warm wash medium.

B. Wash spleen cell suspension 3x by centrifugation in wash medium.

C. Count cells and determine per cent viability.

II. Myeloma Cells

For the day of fusion, myeloma cell suspensions should be at ~10^7cells/ml in wash medium.

III. Preparation Of PEG

PEG is melted in a water bath at 56°C. Prepare a 50% solution of PEG in wash medium and sterilize by filtration through a 0.22 μm filter. Be sure that PEG is not warmer than 37°C when used for fusion.

III. Fusion

A. Combine spleen cells with myeloma cells in varying ratios, such as 2:1 or 3:1 spleen cells : myeloma, then pellet the cell mixture by centrifugation. Remove supernatant.

B. With gentle agitation, slowly add 1-2 ml 50% PEG dropwise to spleen cell-myeloma pellet over a one minute period. Resuspend pellet in 9 ml serum-free culture medium then re-pellet by

centrifugation. Discard the supernatant and resuspend pellet to desired cell concentration in complete culture medium + HAT (diluted 1:50 in complete culture medium).

C. Plate out fusion cultures in 96-well cell culture plates (200 ul/well).

IV. Post-Fusion

A. Approximately seven days post-fusion, feed the fusion cultures by removing half of the medium from each well and adding back complete medium + HAT (diluted 1:50 in complete medium). Hybridoma colonies are usually visible at this time.

B. Approximately 10-14 days post-fusion, supernatant fluids from the fusion cultures are usually ready for testing for presence of antibody.

Note: 50X HT is included in the Fuse-It® kit for use when fusion cultures are cloned by limiting dilution. Cloned cultures are prepared and fed with complete medium + HT (diluted 1:50 in complete culture medium).

Recommended Storage

The components of the Fuse-It® kit may be stored for 3-4 weeks at 4°C provided that these materials remain sterile. For long-term storage, we recommend storing at –20°C. Avoid repeated freeze-thaw cycles.