

QED Fuse-It<sup>®</sup> 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)

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**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 aseptically 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<sup>7</sup> cells/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

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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. HAT solution is prepared by reconstituting lyophilized HAT in 10ml of complete culture medium; this is a 50X solution. Dilute this 50X solution 1:50 in the complete culture medium that is then used to resuspend the pellet of fused cells.

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 prepared as above. 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.

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Note: 50X HT is included in the Fuse-It<sup>®</sup> kit for use when fusion cultures are cloned by limiting dilution. Cloned cultures are prepared and fed with complete medium + HT. HT solution is prepared by reconstituting lyophilized HT in 10ml of complete culture medium; this is a 50X solution. Dilute this 50X solution 1:50 in the complete culture medium that is then used for cloned cultures.

#### **Recommended Storage**

The components of the Fuse-It<sup>®</sup> 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.