TECHNICAL SHEET

Name of Cell Line: **EL4-B5**
Species: **Mouse (*Mus musculus*)**
Type of cell: **Mouse thymoma continuous cell line**

History of the E-4 B5 cells

IL-2 producing EL-4 mouse thymoma cells were sent from the NIH, Bethesda, to the Ludwig Institute for Cancer Research in Epalinges, Switzerland, in 1981. At the Ludwig institute, mutagenesis with ethyl-methane-sulfonate was performed to obtain fusion partners for T cell hybridomas. It was then discovered that the thymidine kinase-deficient and ouabaine-resistant clone EL-4 $Bu^{r}OU^{r}$ 6.1 also activated murine and human B cells via cell contact (1). Subcloning produced the EL-4 B5 cells, which strongly activate the B cells. The publication mentioned that 3 of 8 tested batches of fetal calf serum gave optimal B cell responses with EL-4 B5 cells (2). Various laboratories then reported human B cell culture systems utilizing the EL4 B5 cells; for example, references 3-9. The EL4 B5 cells express CD40-ligand. However, the mechanism for efficient activation of human B cells could not be elucidated; it somehow depends on the batches of fetal calf serum utilized. According to Dr. Rudolf Zubler, many laboratories did not obtain satisfactory results, whereas some laboratories have used EL-4 B5 cells successfully for many years.

Dr. Zubler has retired. But many investigators discover reports, which mention EL-4 B5 cells in conjunction with the generation of human monoclonal antibodies and would like to test the cells. Therefore, availability without restrictions of the EL-4 B5 cells should continue, although the reproducibility of their B cell activating functions is very variable.

Why the cell line is of interest:

The cell line has been used as a feeder layer to generate B cell cultures yielding human monoclonal antibodies.

Number of passages to date: Unknown

Special Permits Needed:

None

Safety level:

Biosafety level 2

Recommended culture medium and subcultivation procedure:

RPMI-1640, 10% FBS heat inactivated, with 2 mM glutamine, usually antibiotics (typically pen/strep or gentamicin plus amphotericin B), 10 mM HEPES, 2-mercaptoethanol (0.056 mM).

Cells should be split regularly.

They can tolerate large splits (volume ratios of 1:10, for example).

Helper function is optimal when cells are in log phase growth.

Keep density below 10^6 per mL at all times.

Special Properties or Characteristics:

B cell helper function depends on the batch of FBS used. There is no simple way to identify optimal FBS lots, other than trial and error with the final assay (typically human B cell expansion).

Helper function may be lost if cells are passaged at densities over 10^6 per mL.

Cryopreservation:

Standard conditions (typically 10% DMSO in serum containing medium).

Selected references

1. Zubler RH, Erard F, Lees RK, Van Laer M, Mingari C, Moretta L, MacDonald HR. Mutant EL-4 thymoma cells polyclonally activate murine and human B cells via direct cell interaction. J Immunol 1985, 134:3662-3668.

2. Li W, Hanvanich M, Werner-Favre C, Brouwers N, Perrin LH, Zubler RH. Limiting dilution assay for human B cells based on their activation by mutant EL-4 thymoma cells: total and anti-malaria responder B cell frequencies. Eur J Immunol 1987, 17:887-892.

3. Lundgren M, Persson U, Larsson P, Magnusson C, Smith CIE, Hammarstrom L, Severinson E. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. Eur J Immunol 1989, 19 :1311-1315.

4. Steenbakkers PGA, Van Wezenbeek PMGF, van Zanten J, The TH. Efficient geneneration of human anti-cytomegalovirus IgG monoclonal antibodies from preselected antigen-specific B cells. Hum Antibod Hybridomas 1993, 4 :166-173.

5. Brinkmann V, Heusser CH. T cell-dependent differentiation of human B cells into IgM, IgG, IgA or IgE plasma cells: High rate of antibody production by IgE plasma cells, but limited clonal expansion of IgE precursors.

6. Matthes T, Werner-Favre C, Tang H, Zhang X, Kindler V, Zubler RH. Cytokine mRNA expression during an in vitro response of human B lymphocytes: Kinetics of tumor necrosis factor-a, interleukin (IL) 6, IL-10 and transforming growth factor b-1 mRNAs. J Exp Med 1993, 178:521-528.

7. De Wildt RMT, Steenbakkers PG, Pennings AHM, van den Hoogen FHJ, van Venrooij WJ, Hoet RMA. A new method for the analysis and production of monoclonal antibody fragments originating from single human B cells. J Immunol Methods 1997, 207:61-67.

8. Leyendeckers H, Odendahl M, Lohndorf A, Irsch J, Spangfort M, Miltenyi S, Hunzelmann N, Assenmacher M, Radbruch A, Schmitz J. Corelation analysis between frequencies of circulating antigen-specific IgG-bearing memory B cells and serum titers of antigen-specific IgG. Eur J Immunol 1999, 29:1406-1417.

9. Weitkamp J-H, Kallewaard N, Kusuhara K, Feiglstock D, Feng N, Greenberg HB, Crowe JE Jr. Generation of recombinant human monoclonal antibodies to rotavirus from single antigen-specific B cells selected with fluorescent virus-like particles. J Immunol Methods 2003, 275:223-237.