Signosis
Innovative Plate Assay Solutions

JAK2 Stable Cell Lines (For Research Use Only)

SL-0200 Jak2 WT Expressing Ba/F3 Cell Line (Hygromycin resistance)
SL-0201 Jak2 V617F Expressing Ba/F3 Cell Line (Hygromycin resistance)
SL-0202 Jak2 V617F + EPOR expressing Ba/F3 cell line (Hygromycin and G418 resistance)

Introduction

Janus Kinases (JAKs) are a family of non-receptor protein tyrosine kinases that mediate the signaling of a wide range of cytokine receptors and hormones, including interleukins, interferons, growth hormones and erythropoietin (EPO). Signaling of cytokine receptors during hematopoiesis is mediated through tyrosine kinase activity of JAK2. The single JAK2V617F mutation in most myeloproliferative neoplasms (MPNs), patients This mutation, commonly found in myeloproliferative neoplasms (MPNs), results in constitutive activation of JAK2, leading to dysregulated signaling cascades and aberrant cell proliferation.

Product description

Signosis has developed human JAK2 expressing Ba/F3 cell line by electroporating parental Ba/F3 cells with a vector containing the human JAK2 gene under an MSCV promoter along with GFP and hygromycin resistance. SL-0202 was also cotransfected with EPOR expression vector containing G418 resistance. Following transfection, clones with resistance to hygromycin (and G418 for SL-0202) were subsequently screened for GFP expression, with protein expression tested via western blot. The clone with the highest expression were selected and expanded to produce these stable cell lines.

Materials provided

One vial of 2-3 x 10^6 cells, in Freezing Media. **IMPORTANT**: store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Handling cells upon arrival

It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.**

IMPORTANT: Please thaw and culture the cells upon arrival**. Also, an adequate number of frozen stocks must be made from early passages as cells will undergo genotypic changes. Genetic instability in transfected cells will results in a decreased responsiveness over time in normal cell culture conditions.

Required Cell Culture Media

- **Complete Growth Media**
  In 450mL of RPMI medium, add 50mL FBS (10% final), and 5mL Penicillin/Streptomycin (1% final).

  - **IL3 instruction:**
  - **For SL-0200 and SL-0201**, add Murine IL3 to medium at a final concentration of 10ng/ml.
  - **For SL-0202,** adding IL3 is optional.

- **1x Freezing Media**
  Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Materials required but not provided

(Can be substituted with a comparable third-party product)

- RPMI-1640 Medium (RPMI)) -- *Hyclone P/N SH30027.01*
- Fetal Bovine Serum (FBS) -- *Fisherbrand P/N 03-600-511*
- Penicillin/Streptomycin -- *Hyclone P/N SV30010*
- Murine IL-3—*Peprotech P/N 213-13*
- DMSO -- *Sigma P/N D8418*

Initial Culture Procedure

1. Quickly thaw cells in a 37°C water bath with careful agitation. Remove from the bath as soon as the vial is thawed.
2. Transfer cells to a T-25cm^2 flask (or 100mm^2 dish) containing 8-12ml of **Complete Growth Media.**
3. Gently rock the flask to ensure the cells are mixed well in the media. DO NOT PIPET.
4. Place the flask with cells in a humidified incubator at 37°C with 5% CO_2.
5. After this incubation time (wait at least 6 hours to overnight), **replace media** with fresh **Complete Growth Media**.

Signosis, Inc. • 1700 Wyatt Drive Suite 10-12 • Santa Clara, CA 95054 • Tel 408 747 0771 • Fax 408 864 2182 •
info@signosisinc.com

Subculture Procedure

1. Subculture/passage cells when density reaches 0.8-1x10^6/ml
   Note: During the time that cells are not used for the experiment ideally, they can be maintained in Complete Growth Media with 50-100µg/ml of Hygromycin B.
   For SL-0202, the cells can be maintained in Complete Growth Media with 50-100µg/ml of Hygromycin B and/or 200-400µg/ml G418.
2. Passage cells every 3 days by inoculating 5x10^5 or in 1:3 to 1:5 ratio with warm Complete Growth Media.

NOTE: Stable cell lines may exhibit a slower proliferation rate compared to parental cells. Do not seed cells at suboptimal density as this may hinder cell growth and division.

Preparing frozen stocks

This procedure is designed for 100mm^{2}dish or T75cm^2 flask. Scale volumes accordingly to other vessels.

1. When cells reach 1x10^6/ml, freeze down cells.
2. Centrifuge culture at 1000 RPM for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media. Resuspend cells at a density of 3x10^6cells/ml in freshly prepared 1X freezing media and gently resuspend by pipetting up and down.
4. Aliquot 1ml cells into a cryogenic vial.
5. Place the cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store it at -80ºC freezer overnight.
6. Transfer cells to liquid nitrogen for long-term storage.

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