

Ba/F3 ETV6-SYK Cell Line

Cat. No: KC-1051

Version 19092002

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I. Cell Line Information

Catalog number	KC-1051
Cell line name:	Ba/F3 ETV6-SYK Cell Line
Gene ID/Accession #:	NM_001135052.4
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous ETV6-SYK fusion protein.
Quantity:	One vial of frozen cells (5X10 ⁶ per vial)
Stability:	Stable in culture over a minimum of 10 passages
Application:	Drug screening and biological assays
Freeze medium:	70% RPMI-1640 + 20% FBS + 10% DMSO
Propagation medium:	RPMI 1640 + 10% FBS
Selection marker:	Puromycin
Morphology:	Mostly single, round (some polymorph) cells in suspension
Subculture:	Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10 ⁵ cells/ml
Incubation:	37 °C with 5% CO ₂
Storage:	Frozen in liquid nitrogen with 70% medium, 20% FBS and 10% DMSO
Doubling time:	Approximately 20 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

II. Background

Spleen tyrosine kinase (SYK) is a nonreceptor tyrosine kinase involved in signal transduction in a variety of cell types; it associates with different receptors on the surface of various cells such as B cells, mast cells, monocytes, macrophages, and neutrophils, and even osteoclasts and breast cancer cells. SYK is expressed ubiquitously in immune and nonimmune cells and has a much wider biological role than previously recognized. Several studies have highlighted SYK as a key player in the pathogenesis of a multitude of diseases. Inhibition of SYK activity has been explored as a therapeutic option in several allergic disorders, autoimmune diseases, and hematological malignancies.

Ba/F3 cell, a murine interleukin-3 dependent pro-B cell line, is a popular system for exploring both kinases and their inhibitors, because some protein kinases can render the Ba/F3 cells to be depended on the activation of the kinases instead of IL-3 supplement, while their inhibitors can antagonize the kinase-dependent growth effects.

III. Cell Line Generation

Ba/F3 ETV6-SYK cell line was generated using retrovirus vector expressing human ETV6-SYK sequence.

IV. Characterization using western blot

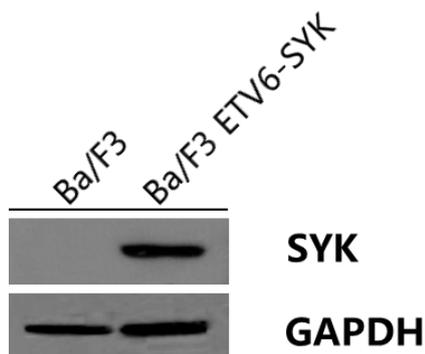
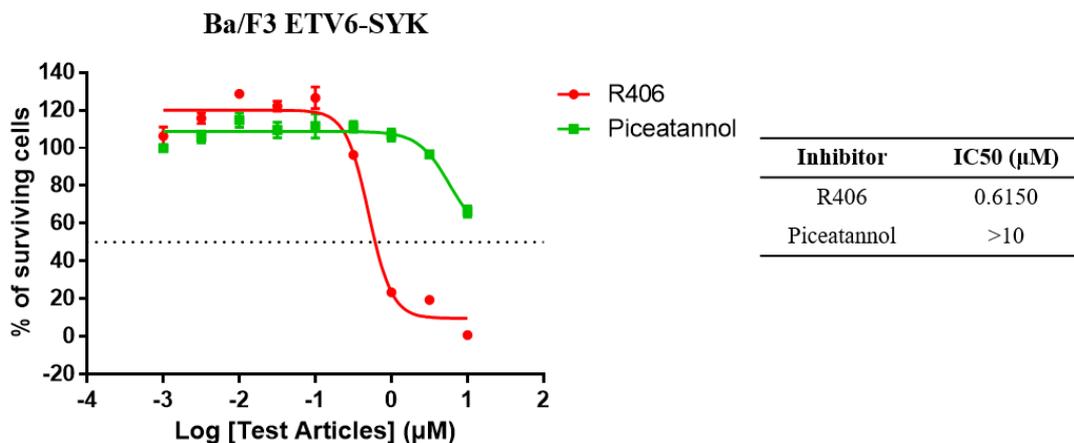


Figure: Characterization of ETV6-SYK overexpressing in Ba/F3 stable clones.

V. Application

- a. Cell-based kinase inhibition screen
- b. Cell viability assay
- c. In vivo efficacy study

Example: kinase inhibitors screening



1. Harvest and seed the Ba/F3 cells in 96-well plate (3000 cells/90ul medium).
2. Next day, add 10ul 10X serially diluted compound solution each well and incubate the plates for another 72 hours.

3. Add 100ul Cell Titer-Glo each well, mixed and readout using Envision.
4. Plot the dose-responsive curve and fit the IC50 (the concentration of 50% inhibition of DMSO vehicle treated clones) using GraphPad Prism software (Version 5).

VI. Cell Resuscitation

1. Prewarm culture medium (RPMI-1640 supplemented with 10% FBS) in a 37°C water bath.
2. Thaw the frozen vial in a 37°C water bath for 1-2 minutes.
3. Transfer the vial into biosafety cabinet, and wipe the surface with 70% ethanol.
4. Unscrew the top of the vial and transfer the cell suspension gently into a sterile centrifuge tube containing 9.0 mL complete culture medium.
5. Spin at ~ 125 x g for 5~7 minutes at room temperature, and discard the supernatant without disturbing the pellet.
6. Resuspend cell pellet with the appropriate volume of complete medium and transfer the cell suspension into a T25 culture flask.
7. Incubate the flask at 37°C, 5% CO₂ incubator.
8. Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10⁵ cells/ml.

VII. Cell Freezing

1. Prepare the freezing medium (70% RPMI-1640 + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Transfer cells to a sterile, conical centrifuge tube, and count the cells.
4. Centrifuge the cells at 250 x g for 5 minutes at room temperature and carefully aspirate off the medium.
5. Resuspend the cells at a density of at least 3 x 10⁶ cells/ml in chilled freezing medium.
6. Aliquot 1 ml of the cell suspension into each cryovial.
7. Freeze cells in the CoolCell freezing container overnight in a -80°C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. Alaa Alhazmi, Spleen Tyrosine Kinase as a Target Therapy for Pseudomonas aeruginosa Infection. J Innate Immun. Published online: June 20, 2018. DOI: 10.1159/000489863
2. Sushma Bartaula-Brevik, et al. Splenic tyrosine kinase (SYK) inhibitors and their possible use in acute myeloid leukemia. Expert Opinion on Investigational Drugs, 2018

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