

Description

Recombinant RPMI 8226 cells constitutively expressing firefly (*Photinus pyralis*) luciferase (BPS Bioscience #79834) have also been engineered to constitutively express the full length human FcRL5 (Fc Receptor Like 5, also known as CD307, ref. seq. NM_031281).

Background

FcRL5 is a single-pass type 1 membrane protein which contains 8 immunoglobulin-like C2-type domains. FcRL5 may be involved in B cell development and lymphomagenesis. Moreover, it is enriched in malignant plasma cells of patients diagnosed with multiple myeloma, and is an attractive target for antibody-drug conjugates (ADC) and anti-FcRL5 CAR T cells. RPMI 8226 cells are human B cells isolated from a plasmacytoma/myeloma patient.

Application

Use as target cells for anti-FcRL5 CAR T cells, anti-FcRL5 ADC, and bi-specific antibodies

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2×10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Parental Cell Line

RPMI 8226, human B cells isolated from a plasmacytoma/myeloma patient, suspension

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 10	BPS Bioscience #79704
Growth Medium 10B	BPS Bioscience #78381

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.



Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO₂. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 10 (BPS Bioscience, #79704):

RPMI 1640 medium supplemented with 10% FBS, 1mM Sodium pyruvate, 1% Non-essential amino acids, 1% Penicillin/streptomycin.

Growth Medium 10B (BPS Bioscience, #78381):

RPMI 1640 medium supplemented with 10% FBS, 1mM Sodium pyruvate, 1% Non-essential amino acids, 1% Penicillin/streptomycin plus 400 µg/ml Geneticin and 0.125 µg/ml puromycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 10 (**no Geneticin or Puromycin**). **Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 10 (**no Geneticin or Puromycin**).
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 10 (**no Geneticin or Puromycin**), and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they reach a density of 2 x 10⁶ cells/ml. At first passage and subsequent passages, use Growth Medium 10B (**contains Geneticin and Puromycin**).

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10⁶ cells/ml, at no less than 0.2 x 10⁶ cells/ml of Growth Medium 10B (**contains Geneticin and Puromycin**). The sub-cultivation ratio should maintain the cells between 0.2 x 10⁶ cells/ml and 2 x 10⁶ cells/ml.

Cell Freezing

1. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10⁶ cells/ml.
2. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
3. Transfer the vials to liquid nitrogen the next day for storage.



Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

A. Validation Data

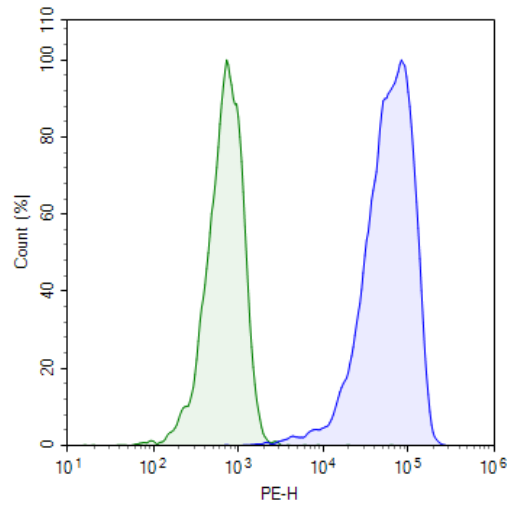


Figure 1. Analysis of FcRL5 expression in FcRL5/Firefly Luciferase RPMI 8226 cells. 35,000 FcRL5/Firefly Luciferase RPMI 8226 cells and parental Firefly Luciferase RPMI 8226 cells (BPS Bioscience #79834) were stained with PE-conjugated anti-human FcRL5 antibody (BioLegend, #340304) and analyzed by flow cytometry. The parental Firefly Luciferase RPMI 8226 cells are shown in green, and the FcRL5/Firefly Luciferase RPMI 8226 cells are shown in blue.

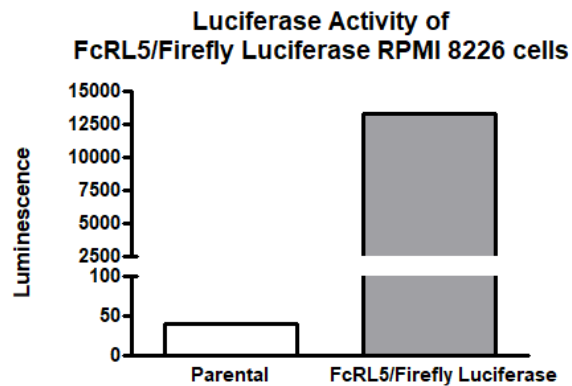


Figure 2. Luciferase activity in the FcRL5/Luciferase RPMI 8226 Cell Line. FcRL5/Firefly Luciferase RPMI 8226 cells or parental RPMI 8226 cells were seeded in a 96-well plate at a density of 25,000 cells/well, and the luciferase activity was measured using the ONE-Step luciferase assay system (BPS Bioscience #60690).

Sequence

Human FcRL5 sequence (NM_031281)

MLLWVILLVLAPVSGQFARTPRPIIFLQPPWTTVFQGERVTLTCKGFRFYSPQKTKWYHRYLGKEILRETPDNILEVQESGEYRCQA
 QGSPLSSPVHLDFFSSASLILQAPLSVFEGDSVVLRCRAKAEVTLNNTIYKNDNVLAFLNKRTDFHIPHACLKDNNGAYRCTGYKESCC
 PVSSNTVKIQVQEPFTRPVLRASSFQPIGPNVTLTLCETQLSLERSDVPLRFRFRDDQTLGLGWSLSPNFQITAMWSKDSGFYWC
 KAATMPYSVISDSPRSWIQVQIPASHPVLTLSPEKALNFEGTKVTLHCETQEDSLRTLRYFYHEGVPLRHKSVCRCERGASISFSLTTE
 NSGNYYCTADNGLGAKPSKAVSLSVTVPVSHPVLTLSAEALTFEGATVTLHCEVQRGSPQILYQFYHEDMPLWSSSTP
 SVGRVFSFSLTEGHSNYYCTADNFGPQRSEVVSFLVTVPVSRIPLTRVPRAQAVVGDLELHCEAPRGSPPILYWFYHEDVTL
 GSSAPSGGEASFNLSTAETHSGNYSCEANGLVAQHSDTISLVIVPVSRIPLTRAPRAQAVVGDLELHCEALRGSSPILYWFYH
 EDVTLGKISAPSGGASFNLSLTTETHSGIYSCEADNGLEAQRSEMVTLKVAVPVSRIPLTRAPGTHAAVGDLELHCEALRGSPIL
 LYRFFHEDVTLGNRSSPSGGASLNLSTAETHSGNYSCEADNGLGAQRSETVTLITGLTANRSGPFATGVAGGLLSIAGLAAGALL
 YCWLSRKAGRKPASDPARSPSDSDSQEPTYHNVPaweELQPVYTNANPRGENVVYSEVRIIQEKKKHAVASDPRHLRNLKGSPIIY
 SEVKVASTPVSGSLFLASSAPHR

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
Firefly Luciferase – RPMI 8226 Recombinant Cell Line	79834	2 vials
FcRL5 CHO Cell Line	78375	2 vials
FcRL5 HEK293 Cell Line	78374	2 vials