

# **Product Sheet**

# H\_MRGPRX2 RBL-2H3 Cell Line

Catalog number: GM-C40579

Version 3.3.1.250718

MRGPRX2 (Mas-related G protein-coupled receptor X2) is a G protein-coupled receptor whose mast cell activation pathway is closely associated with various diseases, such as drug hypersensitivity, chronic urticaria, and chronic inflammation. Currently, MRGPRX2 has become an important research target in the fields of allergy, inflammation mechanisms, and drug risk prediction.

Compound 48/80 can activate MRGPRX2 on the surface of mast cells, leading to an increase in intracellular Ca<sup>2+</sup> concentration and ultimately inducing cell degranulation, resulting in the release of mediators such as histamine and  $\beta$ -hexosaminidase ( $\beta$ -Hexosaminidase,  $\beta$ -Hex). Cortistatin-14 is an endogenous agonist of MRGPRX2, which also binds to and activates this receptor to promote mast cell degranulation. As such, it is widely used as a tool compound in studies investigating the function and signaling pathways of MRGPRX2.

The H\_MRGPRX2 RBL-2H3 cell line is a clonal, stable RBL-2H3 cell line that constitutively expresses the human MRGPRX2 gene, constructed using lentiviral technology. This cell model enables quantitative analysis of mast cell degranulation induced by agonists such as Compound 48/80 and Cortistatin-14 by detecting the release of  $\beta$ -hexosaminidase. It offers an efficient and reliable platform for studying MRGPRX2-related mechanisms of allergy and inflammation, as well as screening potential modulators.



Quantity	5E6 Cells per vial,1 mL		
Product Format	1 vial of frozen cells		
Shipping	Shipped on dry ice		
Storage Conditions	Liquid nitrogen immediately upon receipt		
Target	Human_MRGPRX2		
Gene ID/Uniprot ID	Q96LB1		
Host Cell	RBL-2H3		
Recovery Medium	EMEM(ATCC)+10% FBS+1% P.S		
Growth medium	EMEM(ATCC)+10% FBS+1% P.S+0.25 µg/mL Puromycin		
Note	Cells should be cultured using ATCC/30-2003 EMEM medium or Growth medium from Genomeditech.		
Freezing Medium	90% FBS+10% DMSO		
Growth properties	Adherent		
Growth Conditions	37°C, 5% CO <sub>2</sub>		
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.		
Safety considerations	Biosafety Level 2		
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.		



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## Materials

Reagent	Manufacturer/Catalogue No.
EMEM	ATCC/30-2003
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Puromycin	Genomeditech/GM-040401
APC anti-human MRGX2 Antibody	Biolegend/359006
Compound 48/80	MCE/HY-115768
Cortistatin-14	TargetMol/TP1570
Rat β-Hex Elisa kit	mlbio/ml038039

# Figures



Figure 1 | H\_MRGPRX2 RBL-2H3 Cell Line (Cat. GM-C40579) was determined by flow cytometry using APC antihuman MRGX2 Antibody (Biolegend/359006).





ELISA Rat beta-Aminohexosidase(β-Hex) content Cell Supernatant



Figure 2 |  $\beta$ -Hex Release Assay (ELISA). The H\_MRGPRX2 RBL-2H3 Cell Line (Cat. GM-C40579) was seeded at a density of 2.5 × 10<sup>5</sup> cells per well in a 96-well plate. Cells were stimulated with either Compound 48/80 (C48/80, MCE/HY-115768, 5 µg/mL) or Cortistatin-14 (TargetMol/TP1570, 5 µg/mL) for 1.5 hours. Following incubation, cell supernatants were collected and OD450 was measured using a Rat  $\beta$ -Hexosaminidase ELISA kit (mlbio/ml038039). A standard curve was generated using provided standards, and results are presented as  $\beta$ -Hex concentrations (ng/mL) in the supernatant.

### **Cell Recovery**

#### Recovery Medium: EMEM(ATCC)+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at  $-70^{\circ}$ C. Storage at  $-70^{\circ}$ C will result in loss of viability.

- a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 3 minutes).
- b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately 176 x g for 5 minutes. Discard supernatant.
- d) Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- e) Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

# **Cell Freezing**

Freezing Medium: 90% FBS+10% DMSO

上海市浦东新区康威路 299 号 1 幢东区 505-507 邮编 201315 505-507,5th Floor, East District, Building 1,No.299 Kangwei Road, Pudong New Area, Shanghai 本公司产品仅供科研用途, 严禁用于人体治疗! For research use only!



- a) Centrifuge at 176 x g for 3 minutes to collect cells.
- b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
- c) Aliquot 1 mL into each vial.
- d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

#### Cell passage

Growth medium: EMEM(ATCC)+10% FBS+1% P.S+0.25 µg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- a) Remove and discard culture medium.
- b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- c) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 3 minutes at 37°C).
- Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
  Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
- e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- g) Incubate cultures at 37°C.

#### Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

#### Notes

RBL-2H3 cells exhibit a polygonal adherent morphology when cultured at low density. As the cell density increases, rounded cells begin to appear. The cell density should not exceed 80%, as over-confluence can lead to rounding of the cells and significant detachment.

#### Sequence

#### MRGPRX2 Q96LB1

MDPTTPAWGTESTTVNGNDQALLLLCGKETLIPVFLILFIALVGLVGNGFVLWLLGFRMRRNAFSVYVLSLA GADFLFLCFQIINCLVYLSNFFCSISINFPSFFTTVMTCAYLAGLSMLSTVSTERCLSVLWPIWYRCRRPRHLSA VVCVLLWALSLLLSILEGKFCGFLFSDGDSGWCQTFDFITAAWLIFLFMVLCGSSLALLVRILCGSRGLPLTRL YLTILLTVLVFLLCGLPFGIQWFLILWIWKDSDVLFCHIHPVSVVLSSLNSSANPIIYFFVGSFRKQWRLQQPIL KLALQRALQDIAEVDHSEGCFRQGTPEMSRSSLV

#### **Related Products**

OX40

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H_OX40 Reporter Cell Line	H_OX40 Reporter DDX35TM Cell Line		
Cynomolgus_OX40L CHO-K1 Cell Line	H_OX40 CHO-K1 Cell Line		
H_OX40L CHO-K1 Cell Line	H_OX40L HEK-293 Cell Line		
Anti-H_OX40 hIgG2 Antibody(Ivuxolimab)	Anti-OX40L hIgG1 Reference Antibody(Oxebio)		
Anti-OX40L hIgG4 Antibody(Amlitelimab)	Anti-OX40L hIgG4 Reference Antibody(Amlbio)		
Biotinylated Human OX40L Protein; His-Avi Tag	Cynomolgus OX40 Protein; His Tag		
Cynomolgus OX40L Protein; His Tag	Cynomolgus OX40L Protein; mFc Tag		
Human OX40 Protein; His Tag	Human OX40L Protein; His Tag		
Human OX40L Protein; mFc Tag			
IL-4/IL-13			
IL-4 Reporter Cell Line	IL-4/IL-13 Reporter 293 Cell Line		
IL-4/IL-13 Reporter 293 DDX35TM Cell Line	Cynomolgus_IL4R CHO-K1 Cell Line		
H_IL4R CHO-K1 Cell Line			
Anti-IL-4R hIgG1 Antibody(12B5)	Anti-IL4R hIgG4 Antibody(Dupilumab)		
Anti-IL4R hIgG4 Reference Antibody (Dupbio)			
Cynomolgus IL-4R alpha Protein; His Tag	Human IL-4R alpha Protein; His Tag		
Human IL-4R alpha Protein; mFc Tag			
IL-31			
Cynomolgus_IL-31RA OSMR Reporter Baf3 Cell Line	H_IL-31 Reporter Cell Line		
Cynomolgus_IL31RA CHO-K1 Cell Line	H_IL31RA CHO-K1 Cell Line		
H_IL31RA HEK-293 Cell Line	H_IL-31RA OSMR Baf3 Cell Line		
Anti-IL31 hIgG1 Antibody(mAb33)	Anti-IL31RA hIgG1 Antibody(NA633)		
Anti-IL31RA hIgG2 Antibody(Nemolizumab)	Anti-OSMR hIgG4 Antibody(Vixarelimab)		
c-Kit: SCF			
H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line	Cynomolgus_c-Kit(CD117) GNNK(-) CHO-K1 Cell Line		
H_c-Kit(CD117) GNNK(-) CHO-K1 Cell Line	H_c-Kit(CD117) GNNK(-) HEK-293 Cell Line		
H_c-Kit(CD117) GNNK(+) CHO-K1 Cell Line			
Anti-c-Kit(CD117) hIgG1 Antibody(barzolvolimab)	Anti-c-Kit(CD117) hIgG1 Antibody(briquilimab)		
Anti-c-Kit(CD117) hIgG1 Reference Antibody(barbio)			
Biotinylated Human c-Kit(CD117) Protein; His-Avi Tag	Biotinylated Human SCF Protein; His-Avi Tag		
Cynomolgus c-Kit(CD117) Protein; His Tag	Human c-Kit(CD117) D4-D5 Protein; His Tag		
Human c-Kit(CD117) Protein; hFc Tag	Human c-Kit(CD117) Protein; His Tag		
Human SCF Protein; His Tag	Human SCF Protein; mFc Tag		
MRGPRX2			
H_MRGPRX2 Reporter Cell Line	Tango-H_MRGPRX2 CHO-K1 Cell Line		
Cynomolgus_MRGPRX2 CHO-K1 Cell Line	Cynomolgus_MRGPRX2 HEK-293 Cell Line		
Flag-Rat_Mrgprb3 HEK-293 Cell Line	H_MRGPRX2 CHO-K1 Cell Line		
H_MRGPRX2 HEK-293 Cell Line	Mouse_MRGPRX2 CHO-K1 Cell Line		

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