WILSONWOLF

G-REX®

6M - 2cm² / Well &
6M - 5cm² / Well
6-Well Cell Culture Plates

REF Catalog Number 80120M & 80300M

INSTRUCTIONS FOR USE (IFU)

PRODUCT DESCRIPTION

The G-Rex®6M – 2cm² / Well and G-Rex®6M – 5cm² / Well are single-use, multi-well cell culture plates designed for the expansion and recovery of mammalian cells.

Cells reside undisturbed on the gas permeable membrane and divide until they reach a maximum density or harvest timepoint.

Use	Model	REF	STERILITY
RUO	G-Rex®6M – 2cm² / Well G-Rex®6M – 5cm² / Well	80120M 80300M	Gamma Irradiated; Sterility Assurance Level (SAL) has not been validated

INDICATIONS FOR USE

The G-Rex®6M – 2cm² / Well and G-Rex®6M – 5cm² / Well cell culture plates are intended for expansion and recovery of mammalian cells, preferably suspension cells.

Wilson Wolf Manufacturing LLC makes no claims regarding the performance of this product for clinical treatment or therapeutic applications. It is the responsibility of the end user to assess the suitability of these products for specific applications.

CONTRAINDICATIONS

1. Not suitable for use in conditions outside of those typically intended for the maintenance and expansion of mammalian cells.

WARNINGS

1. This IFU is not a comprehensive reference for the application of use in cell culture protocols.
2. Users should be familiar with appropriate application and aseptic techniques involved in standard cell culture and the G-Rex cell culture platform technology.
3. Do not use if the product is damaged.
4. Do not use the product if there is an apparent breach to packaging integrity.
5. Unauthorized modification and improper use may result in the inability to culture cells
6. Do not freeze the product.

PRECAUTIONS

1. Use care when working with a pipette or similar instruments near the bottom of the G-Rex. The gas permeable membrane may become damaged, resulting in a product leak.
2. Walls of the G-Rex®6M –well plates are slightly opaque; operators may experience difficulty visually verifying the location of pipette tips inside the wells. Take care when aspirating supernatant to avoid accidentally aspirating cells.
3. Do NOT attempt to re-sterilize or reuse the product. All G-Rex products are single-use only.
4. Do NOT over-sanitize workstations or incubator shelves prior to use of G-Rex products. Alcohol or other sanitizing vapors could penetrate the G-Rex membrane and cause cell toxicity.

PREPARATION

- Ensure Media is pre-warmed to 37°C.
- Remove G-Rex Cell Culture Plate from the packaging in a clean environment suitable for standard cell culture.

G-Rex Media Volume Capacity	
Model	Working Volume
G-Rex®6M – 2cm² / Well	20 mL
G-Rex®6M – 5cm² / Well	50 mL

METHODS OF USE

Note: To save time and ensure optimal results, contact technical support at info@wilsonwolf.com for more detailed expansion recommendations, and/or experimental design recommendations.

T CELL EXPANSION IN G-REX

Day 0:

- Create a cell suspension of activated cells at a concentration of 0.5 – 2.0 x 10^6 cells/mL.
- Seed activated cells in desired number of G-Rex wells at a surface density of 0.25 -2.0 x 10^6 cells/cm^2 of G-Rex membrane.
- Fill each well to maximum Working Volume of 10 mL/cm^2 (see G-Rex Volume Capacity table above) with Complete Media including appropriate cytokines and serum if applicable.
- **Note**: Ensure cells and media are well mixed once added to the well. A homogenous cell suspension will result in uniform distribution and settling of cells across the entire gas permeable membrane surface area. This is necessary to achieve maximum cell densities and consistent results.
- **Note for optimal T cell expansion**: Complete Media should be formulated with 3-5% HAB Serum, 10 ng/mL of IL-7 and 10 ng/mL of IL-15. Wilson Wolf recommends the following Media and Cytokine combination:

Supplier	Description	Catalog #
Bio-Techne	GMP Human T Cell Media	CCM038-GMP-1L
Bio-Techne	GMP IL-7	BT-007-GMP
Bio-Techne	GMP IL-15	BT-015-GMP-010

- Place G-Rex in a standard incubator for six (6) to nine (9) days without disturbing the cells or the culture.

Day 7, 8, 9 or 10:

- Harvest cells according to the **HARVESTING** instructions.
- Perform desired cell analysis.
- Cells should reach surface densities of 35-45 x 10^6 cells/cm^2 **without intervention for media feeding or cytokine supplementation**.

T CELL ACTIVATION AND EXPANSION IN G-REX

Day 0:

- Create a cell suspension (PBMC or selected T cells) at a concentration of 0.5 – 2.0 x 10^6 cells/mL.
- Seed cells in desired number of G-Rex wells at a surface density of 0.5 -2.0 x 10^6 cells/cm^2 of G-Rex membrane.

  Note: Each well will contain 0.5-2.0 mL/cm^2 of G-Rex membrane for activation. Ensure cells and media are well mixed once added to the well. A homogenous cell suspension will result in uniform distribution and settling of cells across the entire gas permeable membrane surface area. This is necessary to achieve maximum cell densities and consistent results.
- Add suitable activation reagent at standard concentrations or ratios according to the manufacturer's recommendation.
- Place in standard cell culture incubator overnight.

Day 2 or 3:

- Fill each well to maximum Working Volume of 10 mL/cm^2 (see Volume Capacity table above) with Complete Media including appropriate cytokines and serum if applicable.

  Note: For optimal results, see above Complete Media formulation recommendations (IL-7 & IL-15 each at 10 ng/mL).
- Place in standard incubator for six (6) to nine (9) days without disturbing the cells or the culture.

Day 7, 8, 9 or 10:

- Harvest cells according to the **HARVESTING** instructions.
- Perform desired cell analysis.
- Cells should reach surface densities of 35-45 x 10^6 cells/cm^2 **without intervention for media feeding or cytokine supplementation**.

ACTIVATION, TRANSDUCTION, AND EXPANSION IN G-REX

Day 0:

- Create a cell suspension in suitable Media (PBMC or selected T cells) at a concentration of 0.5 – 2.0 x 10^6 cells/mL.
- Seed cells in desired number of G-Rex wells at a surface density of 0.5 -2.0 x 10^6 cells/cm^2 of G-Rex membrane.

  Note: each well will contain 0.5-2.0 mL/cm^2 of G-Rex membrane for activation. Ensure cells and media are well mixed once added to the well. A homogenous cell suspension will result in uniform distribution and settling of cells across the entire gas permeable membrane surface area. This is necessary to achieve maximum cell densities and consistent results.
- Add suitable activation reagent at standard concentrations or ratios according to the manufacturer's recommendation.
- Place in standard cell culture incubator overnight.

  Note: If starting material is selected T-Cells, Activation and Transduction can proceed simultaneously. Contact technical support at info@wilsonwolf.com for more detailed Day 0 simultaneous Activation and Transduction instruction, and/or experimental design recommendations.

Day 1:

- Mix cells in each well to ensure cells are evenly distributed in the media, and obtain a cell count to determine virus needed to achieve desired MOI.
- Add virus and choice of transduction enhancer (optional) at desired MOI to each well.
- Mix cells and virus to ensure sufficient distribution of cells and virus within the media.
- Place in standard cell culture incubator overnight.

  Note: Depending on starting cell seeding surface densities and concentrations, it may be advisable to activate for up to 2 days.

Day 2 or 3:

- Fill each well to maximum Working Volume of 10 mL/cm^2 (see Volume Capacity table above) with Complete Media including appropriate cytokines and serum if applicable.

  Note: For optimal results, see above Complete Media formulation recommendations (IL-7 & IL-15 each at 10 ng/mL).
- Place in standard incubator for six (6) to nine (9) days without disturbing the cells or the culture.

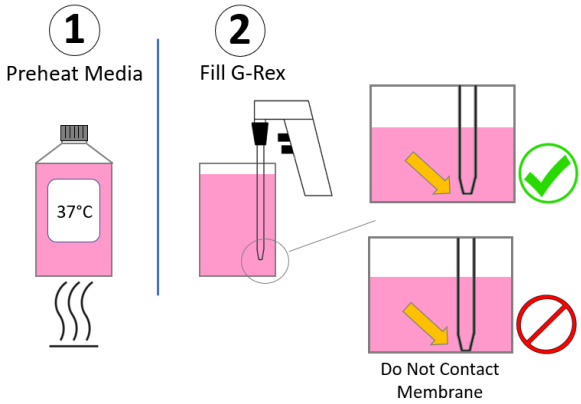
  Note: To save time and ensure optimal results, contact technical support at info@wilsonwolf.com for more detailed activation and expansion recommendations, and/or experimental design recommendations.

Day 7, 8, 9 or 10:

- Harvest cells according to the **HARVESTING** instructions.
- Perform desired cell analysis.
- Cells should reach surface densities of 35-45 x 10^6 cells/cm^2 **without intervention for media feeding or cytokine supplementation**.

STANDARD G-REX OPERATIONS

1. Ensure the media is pre-warmed to 37°C.
2. Fill G-Rex – G-Rex is optimally designed with 10 mL/cm^2 capacity. To optimize and simplify cell manufacturing operations, use the entire media capacity of G-Rex. There is no need for media exchanges, feeding, or cytokine supplementation for most applications.
3. Ensure cells are evenly distributed in media during seeding to ensure uniform settling by gravity on the membrane.
4. For cell counts in process, to avoid accidental aspiration and inaccurate counts, carefully aspirate 50% of the media volume taking care not to disturb the cell layer. Carefully re-suspend the cells in the remaining volume by pipetting up and down, then measure the volume and sample for counts.
5. Alternatively, carefully harvest the remaining volume into a conical tube, rinse the well, re-suspend, measure the volume, and sample for counts.



HARVESTING

Remove (Reduce) excess Waste Media by volume reducing each well to ~4-5 mL/cm^2. Keep pipette away from the bottom of G-Rex well to avoid removing cells or contacting the membrane.

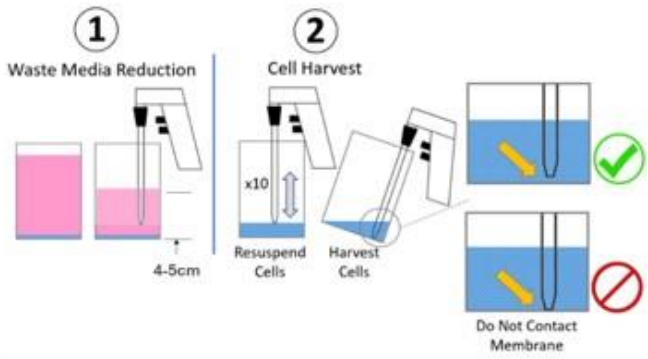
Waste Media Reduction Volumes:

Model	Waste Media Reduction Volume	% of Working Volume Removed	Approx. Pipette Distance from Membrane
G-Rex®6M – 2cm² / Well	10 mL	50%	5cm
G-Rex®6M – 5cm² / Well	25 mL	50%	5cm

Using the remaining media, resuspend the cells by pipetting vigorously (up and down, x10) making sure to wash the sides of the well to ensure all cells are resuspended.

Tilt G-Rex and remove cells using a pipette.

Use care when working with a pipette or similar instruments near the bottom of the G-Rex Well Plate. The gas permeable membrane can be damaged resulting in a product leak.



Contact info@wilsonwolf.com for more detailed instructions for other cell culture or cell manufacturing applications including but not limited to:

- Tumor Infiltration Lymphocytes (TIL)
- Natural Killer cells (NK)
- CAR-NKs
- CAR-T cells
- Tregs
- Hematopoietic Stem Cells (HSCs)
- Red Blood Cells (RBCs)

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Patent: www.wilsonwolf.com/patents
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