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TheraPEAK® X-VIVO® Media Systems

General information

Contents:

I. Introduction

TheraPEAK® X-VIVO® Media is a high-performing media series providing nutritionally adequate and balanced environments for a variety of hematopoietic cells including:

- T cells
- CAR-T cells
- Peripheral blood lymphocytes (PBL)
- Tumor infiltrating lymphocytes (TIL)
- Human monocytes and macrophages
- Lymphokine-activated killer cells (LAK)
- Hematopoietic stem cells (HSCs)
- Dendritic cells (DCs)

Our scalable media are widely cited in scientific publications and proven to reliably work in many cell therapy applications around the world, from research stages to clinical trials to FDA-approved cell therapies.

TheraPEAK® Products are manufactured according to GMP standards and can be safely and confidently used in your clinical processes. Our manufacturing sites are FDA registered with an ISO 13485 certified quality management system.

II. TheraPEAK® X-VIVO® Varieties

TheraPEAK® X-VIVO® 10 Media are optimized for slower growing, less mature cells, such as hematopoietic stem cells.

TheraPEAK® X-VIVO® 15 Media are most appropriate for rapidly growing cells, specifically cells of the immune system.

TheraPEAK® X-VIVO® 10 Media and 15 Media are also available with recombinant transferrin instead of native human transferrin.

III. Features and benefits

- Reduced variables with serum-free formulation
- Multiple formulations allowing seamless transition into customer-specific processes
- Custom packaging available to meet scale-up needs
- Easy-to-use formulation only requiring the addition of cytokines

IV. Ordering information

Additional formulations are available upon request. Please contact Scientific Support for further information.

TheraPEAK® X-VIVO® Medium with native transferrin			
Cat. no. NA	**Cat. no. EU**	**Product**	**Size**
BP04-743Q	BEBP04-743Q	TheraPEAK® X-VIVO® 10 Medium without gentamicin and phenol red, contains native transferrin	1 L bottle
BP04-744Q	BEBP04-744Q BEBP02-061Q	TheraPEAK® X-VIVO® 15 Medium with L-glutamine, without gentamicin and phenol red, contains native transferrin	1 L bottle
08-879H 08-879P1	BE08-879H BE08-879P1	TheraPEAK® X-VIVO® 15 Medium with L-glutamine, without gentamicin and phenol red, contains native transferrin	5 L bag 1 L bag
TheraPEAK® X-VIVO® Medium with recombinant transferrin			
Cat. no. NA	**Cat. no. EU**	**Product**	**Size**
N/A	BEBP02-055Q	TheraPEAK® X-VIVO® 10 Medium with L-glutamine, without gentamicin or phenol red, contains recombinant transferrin	1 L bottle
BP02-054Q BP02-054P1	BEBP02-054Q BEBP02-054P1	TheraPEAK® X-VIVO® 15 Medium with L-glutamine, without gentamicin and phenol red, contains recombinant transferrin	1 L bottle 1 L bag

V. Research use formulations

In addition to our GMP grade media, we also provide an extensive portfolio of research use only (RUO) formulations. Please contact Scientific Support for additional information regarding RUO X-VIVO® Media.

We also offer an extensive portfolio of high quality, research grade primary blood and immune cells including mobilized blood, fresh leukopaks, and whole blood.

VI. Storage

TheraPEAK® X-VIVO® Medium should be stored at 2–8°C. Protect from light.

VII. Instructions for use

T-cell expansion

Media preparation

TheraPEAK® X-VIVO® Cell Culture Medium may be supplemented with cytokines such as with the TheraPEAK® AmpliCell® recombinant IL-2, IL-7 or IL-15. The amount of cytokines required may vary depending on the user's application. For standard T cell expansion, it is suggested to use 100 IU/mL of recombinant human IL-2. The medium supplemented with cytokine may be stored at 2–8°C for up to 10 days. When in use, minimize exposure to light.

Guideline for T-cell expansion in a T-flask

For optimal gas exchange in static T-flasks, it is recommended that the medium height be less than 3 mm.

1. Prepare fresh peripheral blood mononuclear cells (PBMCs) or thaw frozen vials of PBMCs in a 37°C water bath according to standard thawing protocols.
   T cells may be isolated from PBMCs for subsequent expansion. A variety of commercial cell separation products may be used to isolated T cells from PBMCs.
2. Wash the cells with TheraPEAK® X-VIVO® Medium (containing cytokine).
3. Centrifuge the cells at 200–300 x g for 5–10 minutes and remove wash buffer.
4. Resuspend the cells in TheraPEAK® X-VIVO® Medium (containing cytokine). Determine viable cell concentration using standard cell counting protocols.
5. Plate the required number of cells into appropriate tissue culture vessel.
   For example, plate 1.0 x 10^6 viable PBMCs or 0.5 x 10^6 viable T cells in 1 mL TheraPEAK® X-VIVO® Medium

(containing cytokine) into a single 24-well plate.

6. Stimulate the T cells for expansion using a variety of commercial anti-CD3 and anti-CD28 T cell activation products as recommended by the supplier.
7. Incubate the culture vessel at 37°C in a humidified incubator with 5% CO_2.
   On day 1 or 2, lentiviral or retroviral transduction can be performed.
8. On day 2 or 3, transfer the cells from 24-well plate into a T-25 flask for expansion.
   Remove the T cell activation product according to the protocols recommended by supplier.
9. Continue T cell expansion by adding fresh TheraPEAK® X-VIVO® Medium (w/cytokine) every 2–3 days and readjust the cell density to 0.5–1.0 x 10^6 viable cells/mL
10. Several hundred to over 1000-fold cell expansion can be achieved in 10–14 days post-seed.
11. Harvest cells when the desired cell number is achieved and proceed to downstream application (e.g. cell analysis).

Dendritic cell expansion/maturation from PBMCs

Adherence medium

Supplement TheraPEAK® X-VIVO® Medium with 0.5% heat-inactivated human plasma containing 50 U/mL heparin. Store at 4°C.

DC culture medium base

Add IL-4 (1000 IU/mL) and GM-CSF (800 IU/mL) to TheraPEAK® X-VIVO® Medium.

NOTE: Once cytokines are added do not store medium for >30 minutes. Cytokines should be added fresh each time medium is used.

Release buffer

Prepare a stock solution of 5 mM EDTA in PBS (w/o Ca^{2+} or Mg^{2+}) by aseptically adding 10 mL 0.5M EDTA to 990 mL PBS. This buffer can be stored at 2–8°C for 1 year.

1. Prepare fresh peripheral blood mononuclear cells (PBMCs) or thaw frozen vials of PBMCs in a 37°C water bath according to standard thawing protocols.
2. Wash the cells with warmed DC culture medium base.
3. Centrifuge cells at 300 x g for 10 minutes at room temperature.
4. Aspirate supernatant without disturbing cell pellet and resuspend pellet in 1–3 mL medium.
5. Determine viable cell concentration using standard cell counting protocols.
6. Plate the required number of cells into appropriate tissue culture vessel.
   For example, plate 5.0 x 10^6 viable PBMCs/ mL into an appropriate flask or well plate using adherence medium.
7. Incubate the flasks/wells/plates at 37°C, 5% CO_2 for 2 hours to allow the cells to adhere. Verify adherence under microscope.

   NOTE: About 10–30% of the cells will adhere at this time.
8. Aspirate off non-adherent cells and rinse by gently adding pre-warmed PBS (w/o Ca^{2+} or Mg^{2+} or EDTA. Repeat wash step.
9. Feed adherent cells with DC culture medium base containing cytokines. Place in incubator for 72 hours. On day 3, collect medium and non-adherent cells and spin at 30 x g for 10 minutes.
10. Resuspend cell pellet in DC maturation medium with KLH Antigen and reseed.
11. Add lipopolysaccharide (LPS) at 1 µL/mL.
12. Incubate the culture vessel with KLH antigen at 37°C, 5% CO_2 for 6 hours.
13. Collect medium and nonadherent cells and spin at 300 x g for 10 minutes.
14. Resuspend pellet in DC maturation medium without KLH antigen and reseed.
15. Add 1 µL LPS. Place back in incubator for 72 hours.
16. Prior to harvest on day 6, ensure that release buffer is ice cold. Remove culture medium and detached cells.
17. Rinse flasks with release buffer, collect and rinse in 50 mL tube.
18. Add more release buffer to each flask and incubate at room temperature for 10 minutes.
19. Remove attached cells by tapping sides of flask/plate and pipetting release buffer up/down. Remove all detached cells and collect into a conical tube. Centrifuge at 200 x g for 15 minutes.
20. Proceed to downstream application.

VIII. Contact us

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IX. Product use statement

All X-VIVO® products are produced according to applicable GMP standards and follow the USP/EP guidance for cell and gene therapy raw materials. Lonza Group Ltd. and its affiliates (collectively and individually, "Lonza") make efforts to include accurate and up-to-date information. However, Lonza makes no representations or warranties, express or implied, including as to accuracy or completeness of information. All trademarks belong to Lonza, and are registered in the USA, EU and/or CH, or used in common law, or belong to third-party owners and are used for only informational purposes. All third-party copyrights have been reproduced with permission from their owners. The user bears the sole responsibility for determining the existence of any third-party rights and obtaining any necessary licenses and approvals. For more information, including regarding legal disclaimers, Lonza's intellectual property rights, and how Lonza collects, uses and protects personal information: www.lonza.com/legal, https://www.lonza.com/about-us/strategy/intellectual-property, and www.lonza.com/privacy.