X-VIVO® reference guide 2025

Citations list

Culture of hematopoietic stem cells and embryonic stem cells

1. **Melkoumian, Z. *et al.* Synthetic peptide-acrylate surfaces for long-term self-renewal and cardiomyocyte differentiation of human embryonic stem cells. *Nat Biotechnol* 28, 606–610 (2010)**

In this study, the authors introduce synthetic peptide-acrylate surfaces (PAS) that support the long-term self-renewal and differentiation of human embryonic stem cells (hESCs) in chemically-defined, xeno-free medium.

They use **X-VIVO® 10** Medium as the basal medium for culturing hESCs under chemically-defined conditions. X-VIVO® 10 supplemented with growth factors was used for hESCs for both **short term growth assays** assessing their attachment and growth on different PAS surfaces compared to Matrigel and for **long term cultures** with cell viability, morphology, and marker expression monitored over time. X-VIVO® 10 was also used as a base media for cryopreservation.

2. **Loo, J. *et al.* Microfluidic transfection of mRNA into human primary lymphocytes and hematopoietic stem and progenitor cells using ultra-fast physical deformations. *Sci Rep* 11, 21407 (2021)**

This article introduces a novel microfluidic device using Volume Exchange for Convective Transfection (VECT) for efficient and scalable mRNA transfection into human primary cells without adverse effects.

In the study, **X-VIVO® 10** Media is used for culturing and transfecting **hematopoietic stem and progenitor cells** (HSPCs). **X-VIVO® 10** serves as a base medium for **washing, culturing, and transfecting the CD34⁺ HSPCs** in combination with various supplements and inhibitors.

3. **Everette, K. A. *et al.* *Ex vivo* prime editing of patient haematopoietic stem cells rescues sickle-cell disease phenotypes after engraftment in mice. *Nat. Biomed. Eng* 7, 616–628 (2023)**

The study presents a novel prime editing strategy that effectively reverts the sickle-cell disease (SCD) allele to the wild-type HBB gene in human hematopoietic stem cells, demonstrating high on-target efficiency, minimal off-target effects, and significant therapeutic potential without the need for double-stranded DNA breaks or donor DNA templates, thereby offering a promising one-time autologous treatment for SCD.

X-VIVO® 15 Media in this study is used to maintain the culture of **CD34⁺ human hematopoietic stem and progenitor cells (HSPCs)** derived from mobilized human mononuclear cells. The media was supplemented with human stem cell factors (SCF), thrombopoietin (TPO), and Flt-3 ligand to promote the growth and maintenance of the cultured cells.

4. **Wu, Y. *et al.* Highly efficient therapeutic gene editing of human hematopoietic stem cells. *Nat Med* 25, 776–783 (2019)**

The article presents a novel strategy using CRISPR-Cas9 to edit the BCL11A erythroid enhancer in hematopoietic stem cells, leading to increased fetal hemoglobin production, which holds promise for treating severe β-globin disorders like sickle cell disease and β-thalassemia with durable effects.

X-VIVO® 15 Media in this study is used for the initial culture, electroporation and maintenance of the **CD34⁺ HSPCs** before their differentiation into erythroid cells.

Culture of blood immune cells

1. **Hargreaves, B. K. V., Roberts, S. E., Derfalvi, B. & Boudreau, J. E. Highly efficient serum-free manipulation of miRNA in human NK cells without loss of viability or phenotypic alterations is accomplished with TransIT-TKO. *PLOS ONE* 15, e0231664 (2020).**

This article presents a highly efficient method for transfecting primary human natural killer (NK) cells with microRNAs, using the TransIT-TKO transfection reagent, achieving over 90% transfection efficiency while maintaining cell viability and NK cell functionality, advancing the potential for NK cell-based immunotherapy and research

In the article, **X-VIVO® 10** Serum-free media was used to culture **primary NK cells and PBMCs** in an optimized environment free of serum-derived microRNAs, allowing for efficient transfection with microRNAs.

2. **Ding, Y., Tous, C., Choi, J., Chen, J. & Wong, W. W. Orthogonal inducible control of Cas13 circuits enables programmable RNA regulation in mammalian cells. *Nat Commun* 15, 1572 (2024).**

This article introduces the CRISTAL platform, which leverages split Cas13 proteins for inducible, reversible, and scalable RNA regulation, enabling precise and dynamic control of RNA expression in cells, with potential applications in complex gene circuits, transcriptome engineering, and RNA therapeutics.

In this study, **X-VIVO® 15** Media was used as the base medium for culturing **PBMCs**. The medium was supplemented with 5% human AB serum, N-acetyl L-Cysteine, 2-Mercaptoethanol, and IL-2 to support the growth and activation of PBMCs.

3. **Weinberg, Z. Y. *et al.* De novo-designed minibinders expand the synthetic biology sensing repertoire. *eLife* 13, (2024).**

The article introduces de novo-designed protein minibinders, such as the LCB1 minibinder targeting the SARS-CoV-2 Spike protein, as versatile antigen sensors for synthetic receptors. It demonstrates their potential in various synthetic receptor systems, such as SNIPRs and CARs, by showing their ability to detect viral targets and activate immune responses in engineered cells, suggesting minibinders could significantly enhance synthetic biology applications and cell-based therapies.

After isolating **primary human $CD8^+$ T cell**s from donor blood, the authors cultured the T cells in a medium composed of **X-VIVO® 15** Media, 5% human AB serum, and 10 mM neutralized N-acetyl L-Cysteine, supplemented with 30 units/mL of IL-2.

4. **Mowery, C. T. *et al.* Systematic decoding of cis gene regulation defines context-dependent control of the multi-gene costimulatory receptor locus in human T cells. *Nat Genet* 56, 1156–1167 (2024)**

The study employs large-scale CRISPR interference screens in primary human T cells to functionally map the regulatory elements and trans-acting factors governing the expression of adjacent immune costimulatory genes CD28, CTLA4, and ICOS on chromosome 2q33.2, revealing the complex regulatory logic and interactions that control immune responses and their implications for autoimmune diseases.

The **X-VIVO® 15** Media, in this study, is used to culture **$CD4^+CD127^{lo}CD25^+$ T_{reg} cells and $CD4^+CD25^-$ T_{conv} cells** as well as **$CD4^+$ T cells**.

5. **Cimen Bozkus, C., Blazquez, A. B., Enokida, T. & Bhardwaj, N. A T-cell-based immunogenicity protocol for evaluating human antigen-specific responses. *STAR Protoc* 2, 100758 (2021)**

This protocol describes a rapid, sensitive, and high-throughput method for expanding and detecting antigen-specific T cells from human PBMCs using peptide stimulation and flow cytometry, aiding in the study of T-cell immunity and identification of therapeutic targets.

In the protocol, **X-VIVO® 15** Media is utilized for thawing, resuspension and seeding of **PBMCs**. It is the base medium for cytokine addition for development of distinct dendritic cell subsets that are important for **T-cell activation**. It also acts as a base for the feeding solution to support **T-cell expansion** as well as **re-stimulation.**

6. **Li, H.-S. *et al.* High-performance multiplex drug gated CAR circuits. *Cancer Cell* 40, 1294-1305.e4 (2022)**

The article presents the development of Versatile ProtEase Regulatable CAR (VIPER CAR) systems, which leverage the NS3 protein from Hepatitis C Virus to create controllable CAR T cells that can be safely regulated *in vivo* using FDA-approved drugs, thus improving efficacy and managing side effects like cytokine release syndrome in cancer immunotherapy.

In this study, **T cells (both $CD4^+$ and $CD8^+$)** are cultured in **X-VIVO® 15** Media supplemented with human AB serum, N-acetyl L-Cysteine, 2-Mercaptoethanol, and interleukin-2 (IL-2) after being isolated from whole peripheral blood obtained from healthy donors.

7. Dannenfelser, R. *et al*. Discriminatory Power of Combinatorial Antigen Recognition in Cancer T Cell Therapies. *Cell Syst* 11, 215-228.e5 (2020)

The study proposes the use of combinatorial antigen recognition in chimeric antigen receptor (CAR) T cell therapies to improve the precison of tumor targeting and reduce off-target toxicity. By leveraging multiple antigen inputs using Boolean logic gates, the study identifies dual and triple antigen combinations across 33 tumor types that significantly outperform single antigen recognition, showing enhanced tumor-versus-normal tissue discrimination.

In this study, after isolation and thawing of the primary **CD4⁺ and CD8⁺ T cells**, the cells were cultured in **human T cell medium**. This medium consisted of **X-VIVO® 15 Media**, supplemented with 5% Human AB serum, 10 mM neutralized N-acetyl L-Cysteine, 30 units/mL IL-2.

8. Schmitt, J. *et al*. Repurposing an endogenous degradation domain for antibody-mediated disposal of cell-surface proteins. *EMBO Rep* 25, 951–970 (2024).

In this study, the authors introduce PACTAC, a novel therapeutic strategy that fuses the PCSK9 C-terminal domain with antibodies to target and degrade transmembrane proteins through endolysosomal pathways, expanding the potential of antibody-mediated protein degradation.

The authors use **X-VIVO® 15** Media to maintain primary **CD14⁺ monocytes**. The medium is also used when they infect monocytes with viral isolates and in the reactivation assays.

9. Marcarian, H. Q. *et al*. Renal cancer cells acquire immune surface protein through trogocytosis and horizontal gene transfer. *bioRxiv* 2024.08.07.607036 (2024) doi:10.1101/2024.08.07.607036

The article explores the role of trogocytosis in renal cell carcinoma (RCC), demonstrating that tumor cells acquire immune markers and genomic material from infiltrating lymphocytes, potentially altering their phenotype and contributing to immune evasion.

The authors used **X-VIVO® 15** Media to culture **primary human T cells** which were later cocultured with RCC cell lines.

10. Qiu, F. *et al*. Priming with LSD1 inhibitors promotes the persistence and antitumor effect of adoptively transferred T cells. *Nat Commun* 15, 4327 (2024)

The article shows how targeting the epigenetic regulator LSD1 with pharmacological inhibitors during T-cell activation can alleviate T-cell exhaustion, enhancing the persistence and antitumor efficacy of adoptively transferred T cells in cancer therapy.

The authors use **X-VIVO® 15** Media to culture **human peripheral blood mononuclear cells (PBMCs)**. Additionally, **X-VIVO® 15 Media** was used in the **generation of CD19-CAR T cells**, where pre-activated PBMCs were transduced with lentiviral particles to express the CD19-CAR and **expanded in X-VIVO® 15 Medium** with 50 ng/mL IL-2 for several days before *in vitro* assays or adoptive transfer into tumor-bearing NCG mice.

11. Foss, D. V. *et al*. Peptide-mediated delivery of CRISPR enzymes for the efficient editing of primary human lymphocytes. *Nat. Biomed. Eng* 7, 647–660 (2023)

The article introduces Peptide-enabled RNP delivery for CRISPR engineering (PERC), a highly efficient and non-toxic method for delivering CRISPR ribonucleoproteins into primary human T cells.

In the article, **X-VIVO® 15 Media** was used for **culturing T cells, and NK cells** after isolation.

12. Doglio, M. *et al*. Regulatory T cells expressing CD19-targeted chimeric antigen receptor restore homeostasis in Systemic Lupus Erythematosus. *Nat Commun* 15, 2542 (2024).

The authors describe a therapeutic strategy where regulatory T cells (Tregs) engineered with a CD19-targeted chimeric antigen receptor (Fox19CAR-Tregs) are used to restore immune system homeostasis in a mouse model of Systemic Lupus Erythematosus (SLE). The Fox19CAR-Tregs suppress B-cell activity, reduce autoantibody production, and alleviate inflammation in affected organs without detectable toxicity, suggesting a promising approach for treating autoimmune diseases like SLE.

They use **X-VIVO® 15** Media as a base medium to culture acute lymphotic leukemia cell line (ALL-CM), as well as **CD4⁺CD25⁻ T cells**. They also use **X-VIVO® 15** Media while transducing activated **T_{reg}, T_{conv}, CD4⁺CD25⁻ T cells** with lentivirus vectors.

13. De Monte, L. *et al.* Pro-tumor Tfh2 cells induce detrimental IgG4 production and PGE2-dependent IgE inhibition in pancreatic cancer. *eBioMedicine* 97, 104819 (2023).

This study investigates the characterization of T follicular helper (Tfh) cell subsets in pancreatic ductal adenocarcinoma (PDAC) and their role in modulating anti-tumor immune responses, with potential implications for improving immunotherapy outcomes.

In the study, **X-VIVO® 20 Media** is used for the culture of **tumor-infiltrating lymphocytes** (TILs). The authors **culture small pieces of tumor fragments** in X-VIVO® 20 Media. This allows the TILs present in the tumor to migrate out into the medium.

14. Platten, M. *et al.* A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature* 592, 463–468 (2021).

The authors use **X-VIVO® 20 Media** to freeze **PBMCs** isolated from patients. They incubate thawed PBMCs in X-VIVO® 20 Media before stimulating for IFNγ ELISpot and analysis of T cell subsets. Moreover, they use X-VIVO® 20 Medium to **co-culture lesion infiltrating leukocytes (LILs) with dendritic cells (DCs)**. They also generated patient-autologous rapidly expanded PBMCs (REP cells). They used **X-VIVO® 15 Media for coculturing PBMCs with REP cells and PBMCs** and downstream of REP cells.

Differentiation of iPSCs, HSCs and blood immune cells

1. McMahon, E. *et al.* Brazilin is a natural product inhibitor of the NLRP3 inflammasome. *iScience* 27, 108968 (2024)

The study identifies brazilin, a naturally occurring isoflavonoid, as a novel inhibitor of the NLRP3 inflammasome, demonstrating its ability to significantly reduce NLRP3 activation in both primary murine macrophages and human microglia, as well as *in vivo* in a mouse model of acute inflammation, thereby suggesting its potential as a therapeutic agent for NLRP3-related diseases.

The authors used **X-VIVO® 15 Media** as base of the culture media f**or differentiating induced pluripotent stem cells (iPSCs) into macrophage precursors** which were further differentiated into microglia-like cells.

2. Lock, R. I., *et al.* Macrophages enhance contractile force in iPSC-derived human engineered cardiac tissue. *Cell Reports* 43, 6, 114302 (2024)

The authors in this study developed an all-iPSC-derived human engineered cardiac tissue (hECT) model that incorporates iPSC-derived macrophages to better mimic native heart tissue and explore the role of resident macrophages in cardiac function, with assessments of molecular phenotypes, inflammatory responses, and cardiac contractility.

The authors used **X-VIVO® 15 Media** as base for a conditioning medium to culture the **hemogenic endothelial cell fraction** after initially differentiating the WTC11 cells (which express CD68-EFYP) into hematopoietic stem and progenitor cells. They maintain these cells in X-VIVO® 15 Media for over 8 weeks with weekly media exchanges. This prolonged culture allows for the **maturation of the cells into a myeloid- and monocytic-specific lineage**. They replate these cells in X-VIVO® 15 Media to achieve further **macrophage differentiation**.

3. Raffo-Romero A., *et al.* A co-culture system of macrophages with breast cancer tumoroids to study cell interactions and therapeutic responses. *Cell Reports Methods*, 4,6, 100792 (2024)

The authors developed an optimized methos for co-culturing human macrophages with breast cancer tumoroids to enhance the complexity of 3D cancer models, allowing for improved study of macrophage-tumor interactions and more accurate drug response evaluations in preclinical cancer research.

They used **X-VIVO® 15 Media** as a base for their **macrophage differentiation medium**. They cultured PBMCs containing monocytes in this medium to allow for the **differentiation into macrophages**.

4. Gutbier, S. *et al.* Large-Scale Production of Human iPSC-Derived Macrophages for Drug Screening. *International Journal of Molecular Sciences* 21, 4808 (2020).

This article describes an improved method for the large-scale production of human iPSC-derived macrophages, which are thoroughly characterized to ensure their suitability for drug screening. The method overcomes previous limitations, providing a high yield of macrophages with functional characteristics such as cytokine release, phagocytosis, and chemotaxis, making it valuable for disease modeling and drug development.

The authors use **X-VIVO® 15 Media** in the **differentiation and culture of induced pluripotent stem cell (iPSC)-derived macrophages**.

5. **Shaikh, N. A., Liu, C., Yin, Y., Baylink, D. J. & Tang, X. 1,25-Dihydroxyvitamin D Enhances the Regenerative Function of Lgr5+ Intestinal Stem Cells *In Vitro* and *In Vivo*. *Cells* 13, 1465 (2024).**

This article investigates the role of locally-synthesized high concentrations of active vitamin D (1,25[OH]2D) in promoting intestinal stem cell (Lgr5+ ISC) functions, aiming to enhance intestinal epithelial repair and potentially offer a novel therapy for inflammatory bowel disease (IBD), while avoiding the systemic risk of hypercalcemia.

The authors use **X-VIVO® 15 Media** for **high-purity Lgr5⁺ ISC culture, generation of gut-homing macrophages from bone marrow** and **culturing dendritic cells (DC 2.4).**

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