

ROBUST CULTURE OF MOUSE HEPATIC ORGANOIDS

HepatiCult™ Organoid Growth Medium (Mouse)

Despite the remarkable regenerative capacity of the liver in vivo, sustained in vitro culture of liver tissues has remained a challenge for researchers. Recently, the development of organoid culture techniques has provided the hepatic research field with a convenient method for sustained maintenance of hepatic cells.^{1,2}

Organoids are self-assembling, three-dimensional (3D) cell cultures that incorporate some of the cell types and key features of the represented organ of origin. Due to the maintenance of proliferating stem and progenitor cells, epithelial organoids can be maintained in culture far beyond what is possible with ex vivo primary cell culture.

Hepatic progenitor organoids are cultured by expanding the progenitor cell population, which is postulated to reside in the hepatic ducts. Compared to some epithelial organoid culture systems, hepatic progenitor cells exhibit a far lower rate of spontaneous differentiation in organoid culture. Hepatic progenitors form spherical organoids that maintain bipotency and can be further differentiated by switching to a differentiation medium.^{1,2}

HepatiCult™ Organoid Growth Medium (Mouse) is a serum-free, defined medium that allows for rapid generation of hepatic progenitor organoids from mouse liver tissue. Organoid cultures may be initiated from hepatic ducts, duct fragments, single cells or cryopreserved organoids and cultured in Corning® Matrigel® domes or in a dilute Matrigel® suspension. Hepatic organoids may be observed as early as 24 hours after plating and can be passaged within a week, providing an ongoing source of hepatic cells.

Hepatic organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) express markers of hepatic progenitor cells, hepatic ducts, as well as low levels of mature hepatocytes. Growth of hepatic progenitors as organoid cultures enables convenient in vitro characterization of the hepatic epithelium in a physiologically relevant system and reduces the need for animal use.

Why Use HepatiCult™?

CONVENIENT. In vitro system for generating organoids within a week.

STEP-BY-STEP PROTOCOL. No injury models, hand-picking of ducts or cell sorting required.

SIMPLE, TWO-COMPONENT FORMAT. Serum-free and defined medium formulation.

FLEXIBLE PROTOCOL. Organoids can be grown from duct fragments or single cells and cultured in matrix domes or suspension.

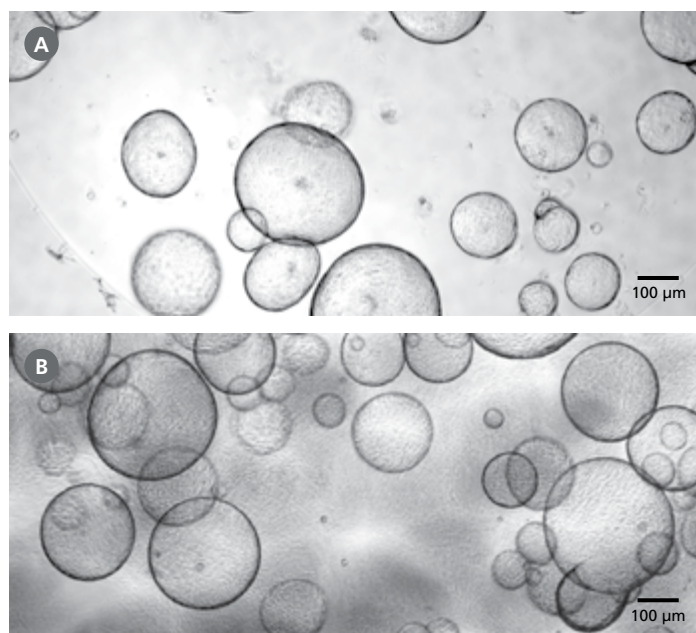


Figure 1. Hepatic Organoids Grown in HepatiCult™ Organoid Growth Medium (Mouse)

HepatiCult™ Organoid Growth Medium (Mouse) supports growth of hepatic progenitor organoids when cultured in (A) Matrigel® domes or (B) a dilute Matrigel® suspension. Organoids were imaged during passage 15 on day 7 of culture.

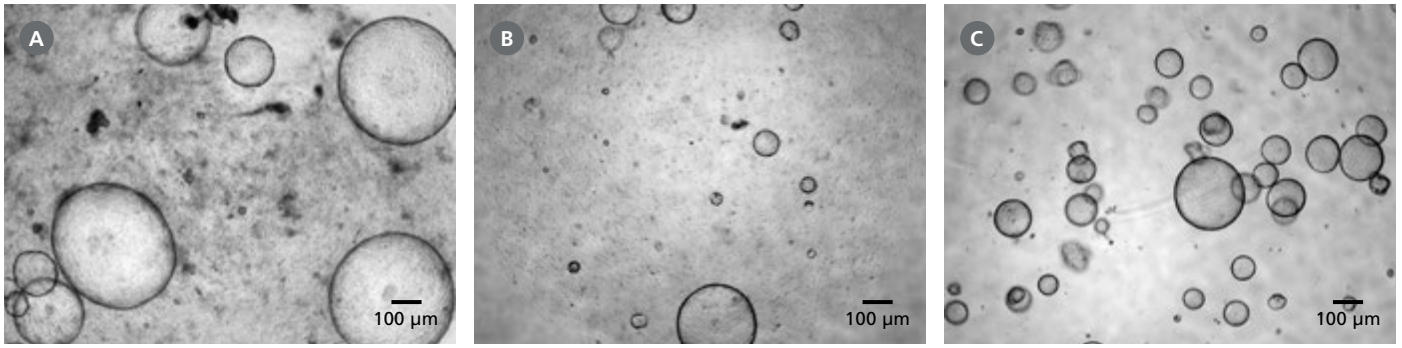


Figure 2. Mouse Hepatic Progenitor Organoids Can Be Initiated from a Variety of Starting Materials.

HepatiCult™ Organoid Growth Medium (Mouse) enables the initiation of hepatic progenitor organoids from (A) duct fragments, (B) single cells or (C) cryopreserved organoids. All organoids were grown in Matrigel® domes and imaged on day 7 of primary culture or the first passage post thaw (cryopreserved organoids).

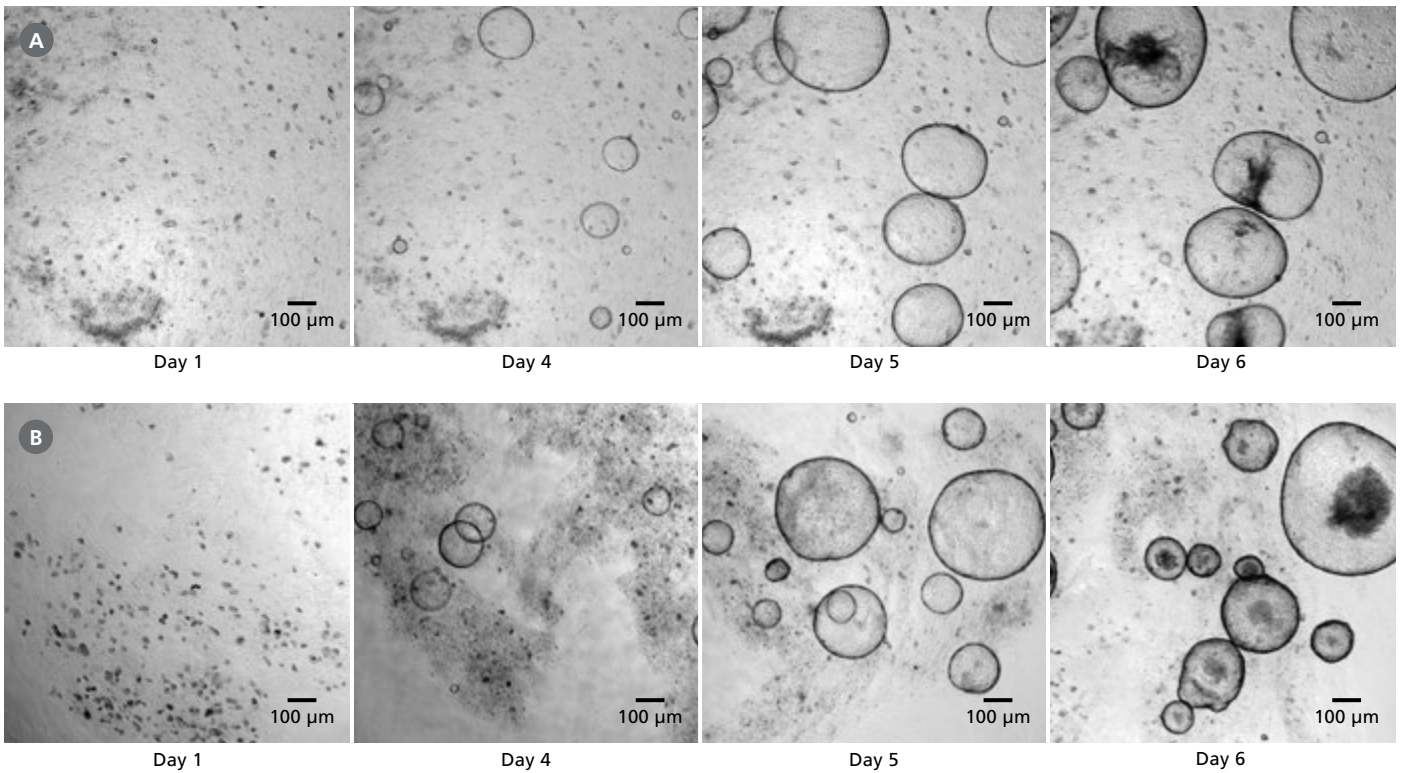


Figure 3. Hepatic Organoids Can Be Grown in Matrigel® Domes or in a Dilute Matrigel® Suspension.

Hepatic progenitor organoids cultured from freshly isolated mouse hepatic duct fragments in HepatiCult™ Organoid Growth Medium (Mouse) can be plated in (A) Matrigel® domes or (B) a dilute Matrigel® suspension. Organoids grown in either culture condition are ready for passage within 4-7 days.

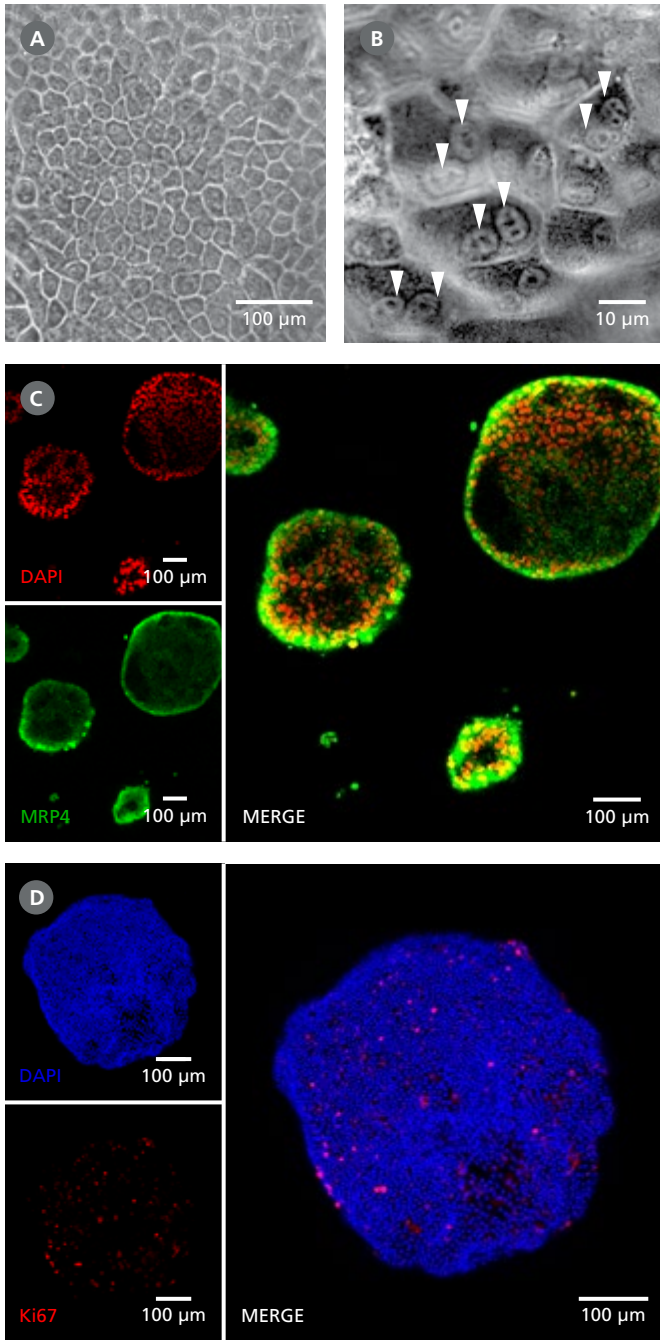


Figure 4. Organoids Grown in HepatiCult™ Organoid Growth Medium (Mouse) Display Some Characteristics Typical of the Mature Hepatic Epithelium.

(A) Hepatic progenitor organoids exhibit the polygonal morphology typical of the hepatic epithelium. (B) Hepatic progenitor organoids show binucleation (arrows), a common feature of mature hepatocytes. (C) Immunocytochemistry analysis shows localization of MRP4 (green), a membrane-bound, unidirectional efflux transporter, along the exterior of the organoids and DAPI (red) localized to the cellular nuclei. This indicates cellular polarization of the organoids with the basolateral surface of the epithelium distal from the lumen. (D) Hepatic organoids contain an actively dividing progenitor population, shown by the expression of Ki67 (red). Cell nuclei are stained with DAPI (blue).

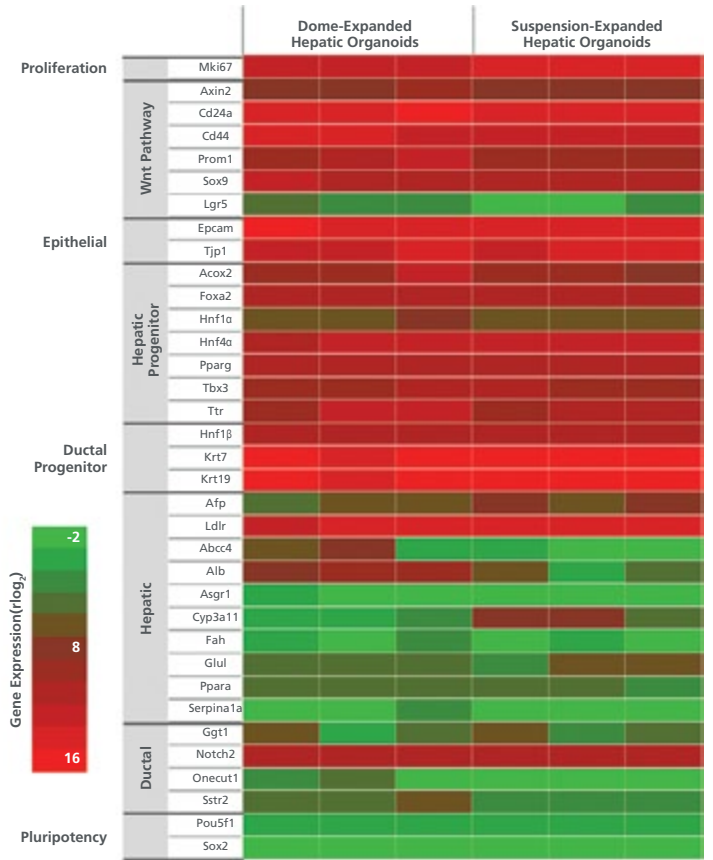


Figure 5. Analysis of Organoid Gene Expression.

Analysis of marker expression by RNA-seq shows organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) in either Matrigel® domes or in a dilute Matrigel® suspension express markers associated with hepatic stem and progenitor cells. The organoids also express low levels of genes associated with mature hepatic cell types, including cholangiocytes and hepatocytes. Columns represent biological replicates at passages ranging from passage 1 - 40.

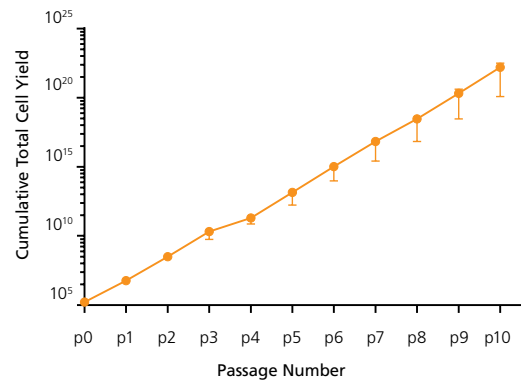


Figure 6. Expansion of Organoids grown in HepatiCult™ Organoid Growth Medium (Mouse).

Organoids cultured with HepatiCult™ Organoid Growth Medium (Mouse) show efficient growth over multiple passages. Cultures were split with an average split ratio of 1:25 at each passage.

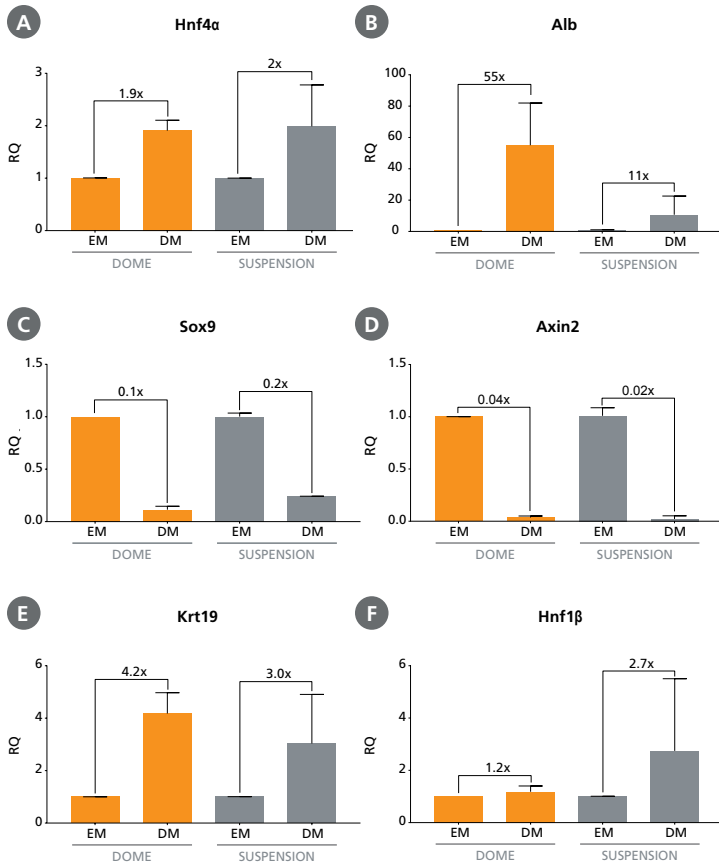


Figure 7. Differentiation of Hepatic Progenitor Organoids.

Hepatic progenitor organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) (EM) can be differentiated to resemble more mature cell types when switched to a differentiation medium (DM). After switching to published differentiation medium,^{1,2} hepatic organoids show the upregulation of mature hepatic markers including (A) Hnf4a and (B) Alb, downregulation of hepatic stem cell and progenitor markers (C) Sox9 and (D) Axin2 and upregulation of ductal markers (E) Krt19 and (F) Hnf1β. Relative quantification (RQ) for each marker is reported relative to 18S and TBP housekeeping genes and normalized with respect to hepatic progenitor organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) and cultured in Matrigel® domes.

Product Information

PRODUCT	CATALOG #
HepatiCult™ Organoid Growth Medium (Mouse)	06030
Mouse Hepatic Organoids	70932
Anti-Adherence Rinsing Solution	07010
Collagenase IV	07909
Dispase	07923
CryoStor® CS10	07930



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SUPPLEMENTARY PROTOCOLS

Read Additional Protocols for Culturing Hepatic Progenitor Organoids
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References

- Huch M., et al. (2013) In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature*. 494(7436): 247-50.
- Broutier L., et al. (2016) Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. *Nature Protocols*. 11(9): 1724-43.