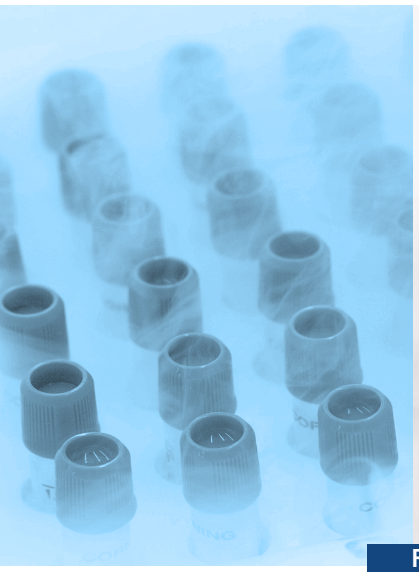
charles river



RESEARCH MODELS AND SERVICES

NCG/PBMC Select Humanization Kit

Advantages

- **Study-ready** – PBMCs have already been pretested for engraftment in the NCG mouse model.
- **Efficient** – Cells have been prescreened, eliminating the time and labor associated with qualification studies.
- **Flexible** – The NCG mouse model can be humanized according to study timelines.
- **Trusted source** – This kit is the product of a partnership between industry leaders Charles River and HemaCare.

Overview

The use of humanized mice has become the standard of study in applications in immuno-oncology and infectious disease research. Peripheral blood mononuclear cells (PBMCs) are of particular interest when humanizing mice. These are adult immune cells that quickly engraft immunodeficient mice. Within 7-10 days, the majority of mature human immune cells present are of human T-cell origin (CD4+ and CD8+) and are ideal for short term studies.

Combined with the NCG mouse (strain code 572), Charles River Laboratories' most immunodeficient model to date, pre-qualified PBMCs will allow researchers to quickly ascertain results. The NCG/PBMC Select Humanization Kit allows researchers to avoid additional work needed to qualify PBMC donor cells. Cell inventory has been screened for engraftment rate, body weight loss, and study term, and cell numbers have been optimized for use in the NCG mouse model.

By providing a "kit" of both cells and mice together, this puts the researcher in control of study timing, a distinct advantage when dealing with a limited therapeutic window (for example, with slow tumor growth rates). The PBMCs are easily transplanted into NCG mice through tail vein injection. In addition, each donor has been chosen to minimize study losses and measurable response while maximizing human immune cell engraftment.

Recommended Protocol*†

1. Enumerate PBMCs and suspend the cells* at $10 \times 10^{\wedge}6$/0.5 mL in sterile PBS.
2. Load 1-cc tuberculin syringes with 25-G × 5/8-in. needles with 0.2 - 0.5 mL cell suspension.
3. Inject 0.2 - 0.5 mL PBMC solution (i.e., $10 \times 10^{\wedge}6$ cells) into the lateral tail vein of recipient NCG mice. PBMC-engrafted mice can be used immediately in experiments.

* *Adapted from* "Animal Models for Autoimmune and Inflammatory Disease," Current Protocols in Immunology **15**, 1-15.21.21, April 2008.

† *Please refer to product insert for precise cell number for injection.*

EVERY STEP OF THE WAY

Example Qualification Study

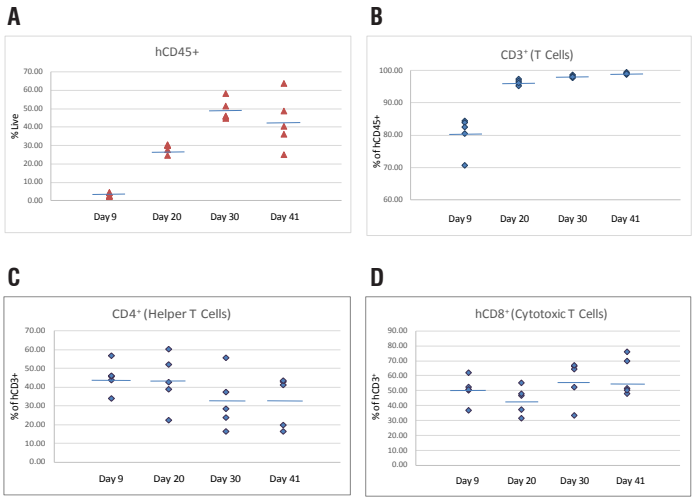
- Groups of 5 NCG mice (age 7-9 weeks) were injected via tail vein with 10 million PBMCs from specific human peripheral blood collections and monitored for a total of 59 days.
- Individual body weights were monitored twice a week over the term of the study.
- Blood samples from individual mice were collected on Days 9, 20, 30, and 41 of the study.
- Any individual animal with a single observation of greater than than 30% body weight loss or three consecutive measurements of greater than 25% body weight loss was euthanized.

Human PBMC Engraftment Example

Charles River NCG mice were successfully engrafted with qualified PBMCs at between 30-60% of total cells with significant T-cell engraftment within the first 1-2 weeks, as shown in Figure 1.

Figure 1: Immune profile of NCG mice humanized with human PBMCs

Dot-plot graphs display the engraftment kinetics of qualified, pretested human PBMCs in the NCG triple-immunodeficient mouse model as determined by flow cytometry analysis. 10 x 10^6 cells were injected on Day 0. The leukocyte marker **(A)** $CD45^+$ and T-cell markers **(B)** $CD3^+$, **(C)** $CD4^+$, and **(D)** $CD8^+$ were evaluated from the day of engraftment until Day 41 of the study, with blood collections on Days 9, 20, 30, and 41. $hCD3^+$ values are relative to $hCD45^+$, while $hCD4^+$ and $hCD8^+$ values are relative to $hCD3^+$. Crossbars are average percent.

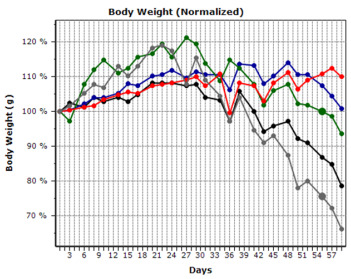


Engrafted NCG Survival Window

As shown in Figure 2, the same study cohorts maintained healthy body weights throughout the 59-day study, allowing NCG/PBMC kit users a 25^+-day window for conducting studies. In a typical experiment, 60-100% survival has been observed.

Figure 2: Study body weights

NCG mice were engrafted with pretested, donor-qualified PBMCs, and the onset of GvHD was followed by tracking body weight changes. This figure shows percent change in body weight compared to starting weight (Day 1) up until 100% of the animals remained in the study (n=5) up to 59 days. Values are actual in gram body weight.



Ordering Information

The standard NCG/PBMC Select Humanization Kit configuration includes ten NCG mouse models and a sufficient number of PBMCs to engraft them. Please contact Customer Service at 1.800.LAB.RATS (1.800.522.7287) with any questions or to place an order. Studies using humanized NCG models are also available from our Discovery Oncology Services Group.

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