



# Taconic's Humanized Immune System Models: Policies, Recommendations, & Resources

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## Policies

### General Policies and Terms:

- Humanized immune system models are provided under our normal [Terms and Conditions for the Sale of Products](#). We are unable to guarantee the performance of humanized immune system models for any specific applications.
- Human Immune System Engrafted Mice (Precision Research Models) orders can be cancelled or changed up to 4 weeks prior to the ship date. Be sure to cancel an order prior to the cut-off time or cancellation fees up to the entire price of the order will be applied. [Click here for more details on Taconic cancellation policies](#).

### Promotional Terms:

- Humanized immune system models associated with discounted promotions are provided under our normal [Terms and Conditions for the Sale of Products](#), unless the specific offering indicates otherwise. We are unable to guarantee the performance of humanized immune system models for any specific applications.
- Taconic Biosciences reserves the right to determine eligibility for discount and to change or discontinue offers at any time without notice.

### Associated Recommendations:

- Before ordering humanized immune system models, investigators should consider how age and the time period since immune system engraftment may impact the model's performance on study.
- Some Taconic promotions grant discounted or free-pilot access to humanized models at advanced ages, with limited availability. We highly recommend mice matching this description be placed on study at the earliest available date. Doing so may help mitigate negative effects associated with age and engraftment duration on the performance of study animals.
- For additional support from a Taconic Scientist, please reach out through the contact form located at the bottom of the [Humanized Mice Webpage](#).

### Associated Resources:

- [Taconic Terms and Conditions for the Sale of Products](#)
- [For-profit Conditions of Use for CIEA NOG® Portfolio Products](#)
- [Non-profit Conditions of Use for CIEA NOG® Portfolio Products](#)

## Recommended Care and Housing for the CIEA NOG Mouse®

### Production at Taconic

The CIEA NOG mouse® (NOG) is severely immunodeficient. Taconic and CIEA recommend the highest level of care possible for this mouse model. Taconic currently produces these mice both in flexible-film isolators at the [Defined Flora](#) health standard using strict gnotobiotic techniques as well as in barriers at the [Excluded Flora](#) and [Opportunist Free™](#) health standards. A current health report may be viewed [online](#).

At Taconic, all items that enter gnotobiotic isolators are steam sterilized, including the feed and drinking water. Mice are housed in polycarbonate ages with wire bar lids (gnotobiotic isolator), polycarbonate cages with wire bar lids and filter tops or individually ventilated cages (barrier production). In barrier settings, all caging components and feed are steam sterilized prior to entering the barrier. For both isolators and barriers, potable groundwater is passed through a series of filters (5 micron, 2.5 micron, 0.2 micron), filled into water bottles and then autoclaved. Barrier cage handling practices are designed to maintain individual cage level biosecurity practices.

### Recommendations for maintenance by users

1. All materials for housing or experimentation ideally should be sterilized by autoclave; alternatively, they may be chemically disinfected or irradiated.
2. Microbiological monitoring should be performed regularly. Testing should include opportunistic agents. The globally harmonized Taconic [International Health Monitoring System™](#) (IHMS™) can supply the confidence needed to work with immunodeficient models.
3. NOG mice should be housed in the cleanest portion of the animal facility. If possible, maintain the NOG mice in their own room or in an immunodeficient mouse room.
4. Personnel movement policies are important to reduce the chance of contamination. The most desirable arrangement is to have dedicated personnel for the NOG mouse room. If separate technicians are not available to care for the NOG mice only, then personnel should enter the room housing the NOG mice prior to going into areas which have a lower health status. They should not return to the NOG mouse room during the same day unless proper personnel decontamination procedures have taken place.
5. Illness or other adverse effects may be linked to infection by opportunistic agents

or excessive stress on the mice. Care should be taken to maintain a high health standard and minimize stress on the mice.

6. As with other immunodeficient models, the NOG mouse may benefit from housing in microisolator cages, such as individually ventilated cages. Using proper decontamination procedures between the changing of cages is recommended. One such approach is to use forceps that are disinfected before use with each new cage to pick up the tail of the mouse.
7. Move animals to a class II laminar flow hood for cage changes and research protocols. Cages can also be changed in HEPA-Filtered animal cage change stations.
8. Irradiation of the NOG mouse should be performed in a sterilized primary container. Mice with scid mutations are radiation sensitive; minimal irradiation is recommended and avoid irradiation if not needed. Please contact Taconic for information on recommended radiation doses and suggested starting points for evaluation of appropriate radiation dose for particular experimental setups.
9. NOG mice are generally non-aggressive and may be group housed, including males.
10. Taconic does not recommend prophylactic antibiotic treatment of NOG or other immunodeficient mice for many reasons, including concerns about increasing antibiotic resistance and intestinal dysbiosis.

Read the related Taconic Bioscience's Insight:

- [Care of Immunodeficient Mice and Rats](#)

### **Tips for acclimation**

NOG mice may have difficulty transitioning to a new diet or water source upon receipt in your facility.

- Taconic feeds NOG mice autoclave sterilized [NIH #31M](#) diet. To assist NOG mice in transitioning to a new diet, mix in some pelleted NIH #31M diet with the new diet. The NIH #31M diet is an open source diet which may be obtained from various vendors.
- Taconic uses water bottles in all NOG husbandry locations. NOG mice may have difficulty transitioning to different types of water bottles or lixits for automatic watering systems. Providing supplementary hydration gel in the bottom of the cage during the acclimation period may prevent dehydration.

Read the related Taconic Bioscience's Insight:

- [Acclimating Research Animals Through Effective Nurturing](#)

[Contact Taconic](#) for any questions regarding the above recommendations. Requirements for care will vary by facility. Please consult your veterinarian or facility manager for more information on working with immunocompromised animals.

## Information on production practices, health testing and health designation for huNOG products

HSCCB-NOG-F, HSCCB-NOG-M, HSCFTL-NOG-F, HSCFTL-NOG-M, HSCFTL-13395-F, HSCCB-13395-F

huNOG and huNOG-EXL products are generated via sublethal irradiation and engraftment of NOG or NOG-EXL mice with human hematopoietic stem cells. They are housed for 10+ weeks post engraftment prior to shipment. QC analysis for huNOG mice occurs at 12 weeks post engraftment, and huNOG-EXL undergo QC analysis at 10 weeks post engraftment.

### Source of mice, housing and procedure locations

The NOG mice used for this engraftment are sourced from one of Taconic's Excluded Flora™ Isolated Barrier Unit production colonies and moved to a semi-rigid isolator at the humanization facility. Mice are removed from the isolator and into a procedure room for irradiation, engraftment and any blood sampling activities. The isolator is sentinelized with SW mice as well as NOG mice, sourced from a Taconic Defined Flora colony. The sentinel program involves weekly exposure of the sentinel mice to soiled bedding and used feed and water from cages within the isolator. Additionally, the sentinel mice accompany the NOG mice during transit to the procedure room and remain in close proximity to the NOG mice during all manipulations within the procedure room; sentinel mice and manipulated NOG mice are transported back to the housing isolator together. The housing isolators are monitored for contamination via modified IHMS™ testing and monthly Opportunist Free™ monitoring. IHMS™ testing is rotated between IHMS-6, IHMS- 13, IHMS-26 and IHMS-52 test panels with IHMS-52 testing occurring once per quarter to allow transit on Taconic trucks. Monthly Opportunist Free™ testing involves pooled fecal samples from both line animal and sentinel cages in addition to oral swab testing. Animals are expected to maintain Opportunist Free™ health status.

### Human cell source and testing

The human hematopoietic stem cells are provided to Taconic as dissected tissue or isolated cells. They have been tested by the vendor and certified negative of hepatitis B, hepatitis C, HIV and LCMV prior to receipt at Taconic. Testing cannot completely guarantee that the HSC material was virus-free. Hence, **THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS** and only used following appropriate handling precautions such as those described in biological safety level 2.

### Basic engraftment procedure description

NOG mice are sublethally irradiated between 4-24 hours prior to IV tail vein injection with human hematopoietic stem cells.

### Quality control

Post-procedure observations include daily examination of mice for health, appearance and injection site healing. Each huNOG and huNOG-EXL mouse is tested by flow cytometry to quantify the level of human leukocytes in peripheral blood.

### Specific notes and recommendations for individual models

**huPBMC-NOG:** NOG mice engrafted with human PBMCs will typically develop Graft vs. Host Disease (GvHD) within 5-7 weeks. GvHD manifests as weight loss, poor clinical condition, infiltration of immune cells into organs and liver damage. PBMC engrafted NOG mice should be monitored closely for condition and be humanely euthanized when found moribund.

**huNOG-EXL:** We have seen that this model engrafts extremely well with chimeric ratios averaging 60% and sometimes over 80%. One of the potential adverse effects we have seen in some animals as a result, is the development of a clinical anemia. Our process improvements have decreased the incidence to 5-10% of mice. Nevertheless, it is recommended that blood sampling is limited to once per 2-week period, and that all animals receive a bolus of subcutaneous fluids when blood samples are collected.

Requirements for care will vary by facility. Please consult your veterinarian or facility manager for more information on working with immunocompromised animals.

### Certificate of Analysis

A complimentary certificate of analysis is provided on the day of shipment for all orders of stem cell engrafted humanized mice. This certificate includes a general overview of the product line, flow cytometry results of cell reconstitution rates collected during quality control testing, and a packing list correlating the tattoo ID of each animal with the data being presented.

All mice engrafted from a single human donor's cells are assigned a Lot number. Lot numbers can be broken down as follows:

HSCCB-13395-20190423-070


Model #                      Week of Engraftment                      Lot ID #

Please note that the date included in the Lot number is the week of engraftment and not the week of birth.

On the packing list included with the animals and within the Certificate of Analysis, one will find a Serial No. column including individual animal identification information. For example:

070 - 001  
 Lot ID #    Serial #

Using the packing list included in the certificate of analysis, correlate the Lot number with the Taconic Transit Carrier number (under the “Box” column of the packing list) to determine which containers housed which donor engrafted animals in transit. huNOG-EXL mice are tattooed at the base of the tail during engraftment. The Animal ID# will match the tattoos on the animals. **Be sure to confirm Animal ID# and container location from packing information provided.**




## TACONIC PACKING LIST

North America: +1 (888) 822-6642  
 Europe: +45 70 23 04 05  
[www.taconic.com](http://www.taconic.com)

<p>Customer Name:            Ship To Customer:            Attention Person:            Sales Order Number: 1261285            Purchase Order Number: 6101496765            Taconic Packing List Number: AL2_20191211_1            Customer Notes:            HUINOEXL Genes Tested: Gene 1 - HCD45 Gene 2 - B Gene 3 - T</p>	<p>Ship Date: 2019-12-11            Model Number(s): HUINOEXL            Nomenclature(s): humanized hGMCsf/hIL3 NOG (line # 13395) live mouse inventory            Source Location(s): IMJ040308C            Courier Reference:</p>
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Box	Section	Qty	Sex	Color	ID	Serial No.	WCE	Genotype	Comment	%hCD45
1	A	4	F	AL		168-022	2019-08-13	(?)		65
			F	AL		168-028	2019-08-13	(?)		64
			F	AL		168-031	2019-08-13	(?)		73
			F	AL		168-040	2019-08-13	(?)		74



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## Example Flow Cytometry protocol for Human Immune System Engrafted Models

### Purpose

This protocol covers the process of flow cytometry immunostaining for assessment of human cell reconstitution (i.e. engraftment efficiency) in human immune system engrafted mouse models.

### Reagents used

Reagent	Vendor	Catalog Number
10X Phosphate Buffered Saline (PBS)	VWR	101076-194
Fetal Bovine Serum (FBS)	Life Technology	10438-026
Sodium Azide	Sigma	S2002
FACS Lysing Solution	BD Bioscience	349202
Stabilizing Fixative	BD Bioscience	338036
Dimethyl Sulfoxide (DMSO)	Sigma	D2650

### Antibody information

Antibody	Antibody Clone	Fluorescent dye conjugate	Vendor	Catalog Number
mCD45	30-F11	PerCP-Cy5.5	BioLegend	103132
hCD45	HI30	PE	BioLegend	304039
hCD3	UCHT1	APC	BioLegend	300439
hCD20	2H7	APC-Cy7	BioLegend	302314
hCD33	P67.6	BV605	BioLegend	366612

## Flow Cytometry Staining Procedures

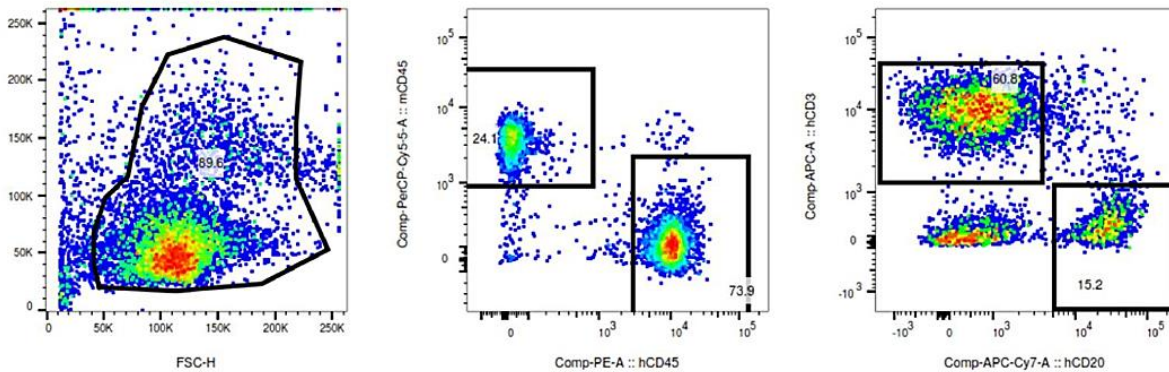
### **\*\*IMPORTANT: KEEP ALL SAMPLES ON ICE THROUGHOUT PROCEDURE**

1. Prepare 100  $\mu$ l volume of anticoagulated whole blood and place samples on ice
2. Prepare antibody dilutions according to manufacturer's recommendations. To ensure proper performance, **it is recommended that antibody reagents be titrated and optimized for use in your own facility. For further information on antibody staining, test validations, and cytometer set-up, consult with antibody manufacturer and/or cytometer manufacturer.**
3. Add the antibody mix to the appropriate sample tubes.
4. Add 90  $\mu$ l of whole blood specimen in EDTA to the labeled tubes.
5. Mix and incubate in the dark on ice for 40 min.
6. Add 2 ml RBC lysis solution to each tube. Brief vortex, then incubate on ice for 7 min in the dark.
7. Spin for 5 min at 1100rpm (200x g) and remove supernatant.
8. Add 1 ml standard wash buffer to each tube and vortex.
9. Spin 5 min at 1100 rpm (200x g) and remove supernatant.
10. Add 500  $\mu$ l stabilizing fixative working solution to each tube, gently vortex and incubate 20 min in the dark.
11. Spin 5 min at 1100 rpm (200x g).
12. Remove supernatant carefully.
13. Wash 2X by adding 1ml standard wash buffer, gently vortex, spin at 1100 rpm for 5 min and remove supernatant.
14. After final wash and supernatant removed, re-suspend cells with 500  $\mu$ l standard wash buffer, mix, and transfer the suspension to labeled polystyrene tubes.
15. Analyze samples on flow cytometer.

## Analysis of flow cytometry data

### Gating Strategy

1. Gate events by plotting SSC-A on y-axis and FSC-H on x-axis.
  - a. Draw gate to include lymphocytes and granulocytes.
  - b. Plot gated events by mouse CD45 vs human CD45.
    - i. The % hCD45+ cells are representative of immune system reconstitution.
  - c. Sub-gate on human CD45+ cells
    1. Plot hCD3 vs hCD20 to evaluate relative percent of T and B cells respectively.



Quality Control data describing peripheral blood hCD45 engraftment are available prior to order completion upon request. Additional peripheral blood data (hCD3 and hCD20) are currently included with the Certificate of Analysis and are not available prior to shipment. For animal welfare purposes, blood collections for flow analyses are conducted once, before the spectrum of human immune cells will have fully reconstituted in the periphery. The timing is sufficient for ensuring adequate peripheral blood humanization (hCD45 guaranteed at or greater than 25%), but is frequently insufficient for allowing other markers, including some within the Certificate of Analysis, to inform animal selection.

## Additional Resources

1. [Taconic Humanized Mice Portfolio](#)
2. [The Taconic NOG Portfolio](#)
3. [huNOG Publication Library](#)
4. [huNOG-EXL Publication Library](#)
5. [huPBMC-NOG Publication Library](#)
6. [Comparison of Cytokine Transgenics for Improved Myeloid Lineage Reconstitution](#)
7. [Taconic Cancellation Policy](#)
8. [Taconic Terms and Conditions for the Sale of Products](#)