Taconic Humanized Immune System Models: Licensing, Care & Resources

Contents

Licensing

Humanized immune system (HIS) mice are sold subject to license restrictions. See the relevant product webpage for details.

Recommended care and housing for NOG Portfolio models including HIS mice

NOG models, especially humanized immune system mice, are very sensitive and require strict attention to proper housing and husbandry. Significant animal morbidity and

mortality can occur if these recommendations are not followed. Consult your facility veterinarian and/or vivarium manager and contact Taconic with any questions prior to ordering.

Production at Taconic

The CIEA NOG mouse® (NOG) and next generation NOG models are severely immunodeficient. Taconic recommends the highest level of care possible for these mouse models, including mice which have been humanized. Taconic currently produces these mice in barriers at the Opportunist Free™ health standard.

At Taconic, all caging components and feed are steam-sterilized prior to entering the barrier. Potable groundwater is passed through a series of filters (5 micron, 2.5 micron, 0.2 micron), filled into water bottles and then autoclaved. In some locations, commercial pre-filled, purified and acidified water bottles are used. 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.
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, NOG mice 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; avoid irradiation if not needed. Chemical myeloablation is another option. Please contact Taconic for information on recommended radiation doses and suggested starting points for evaluation of appropriate radiation dose or chemical myeloablation for particular experimental setups.
    - Read the related Taconic Insight: Considerations for Rodent Irradiation
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 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 Biosciences 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.

Humanized immune system (HIS) mouse production practices and animal health

Source of mice, housing and procedure locations

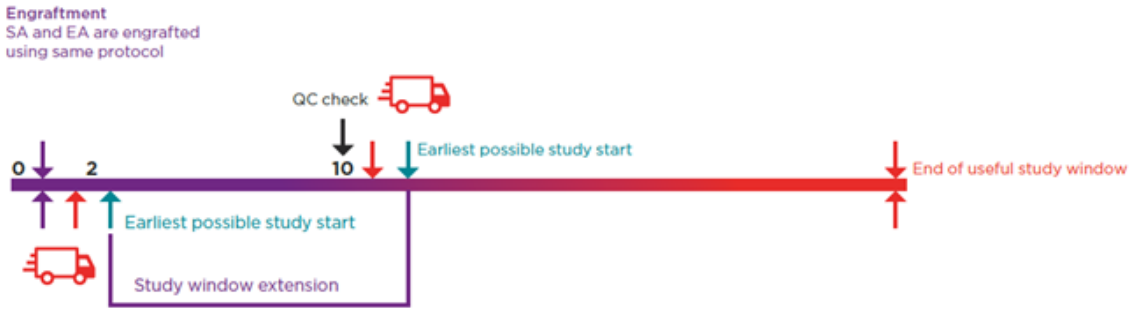
Naïve mice are sourced from a Taconic production colony (Opportunist Free™ health standard) and moved to the humanization facility where they are housed in individually ventilated cages (IVCs). Mice are moved into a procedure room for myeloablation, engraftment and any blood sampling activities. The IVC rack is sentinelized with C3H mice sourced from a Taconic Defined Flora colony, as well as NOG mice. The sentinel program involves weekly exposure of the sentinel mice to soiled bedding. The IVCs are monitored for contamination via modified IHMS™ testing and monthly Opportunist Free™ monitoring. Monthly Opportunist Free™ testing involves pooled fecal samples from both line animal and sentinel cages. Animals are provided to customers at the Opportunist Free™ health standard.

Human cell source and testing

Human hematopoietic stem cells and PBMCs are provided to Taconic as isolated cells. They have been tested by the vendor and certified negative for hepatitis B, hepatitis C, HIV and LCMV prior to receipt at Taconic. Testing cannot guarantee that the human material was virus-free. Taconic handles humanized immune system models as BSL-1 and recommends referring to the biosafety guidelines of the receiving institution for guidance and classification.

Basic engraftment procedure description

Standard Access (SA) and Early Access (EA) timeline comparison



huNOG, huNOG-EXL, FcResolv® huNOG and FcResolv® huNOG-EXL products are generated as follows:

Human cell type	Models	Description	Product details
CD34+ HSC	huNOG huNOG-EXL FcResolv® huNOG FcResolv® huNOG-EXL	5-6 week-old mice undergo myeloablation 4-24 hours prior to intravenous tail vein injection of human hematopoietic stem cells (HSCs)	huNOG, FcResolv huNOG, huNOG-EXL SA (Standard Access) and FcResolv® huNOG-EXL SA mice are housed for 10+ weeks post engraftment (WPE) prior to shipment. huNOG-EXL EA (Early Access) and FcResolv® huNOG-EXL EA mice are shipped within ~2 WPE and do not undergo QC analysis.
PBMC	huPBMC-NOG huPBMC-B2m-NOG	6-8-week-old mice are engrafted with human PBMCs via IV tail vein injection and shipped within ~1 week.	Myeloablation is typically not used prior to PBMC engraftment as it can shorten the study window.

Quality control

Post-procedure observations include daily examination of mice for health, appearance and injection site healing. huNOG, FcResolv® huNOG, huNOG-EXL SA, and FcResolv® huNOG-EXL SA mice are tested by flow cytometry to quantify the level of human leukocytes in peripheral blood. huNOG-EXL EA and FcResolv® huNOG-EXL EA mice are generated using HSCs from a donor previously validated to engraft at or above Taconic's QC threshold. As they ship to customers prior to significant reconstitution of human cells in the periphery, they are not individually QC'd by Taconic.

All animals are checked for general health and condition prior to shipping.

Potential adverse outcomes and animal care

Requirements for care will vary by facility. Please consult your veterinarian or facility manager for more information on working with immunocompromised animals. We recommend you follow IACUC/Oversight Body and Guide for the Care and Use of Laboratory Animals for blood volume sampling by animal weight. Carefully evaluate animal condition following blood sampling, as subcutaneous fluids and/or palliative care may be required.

When performing clinical evaluation of immunodeficient models, please remember to be mindful of over-handling, as these actions can elevate stress levels and have an adverse impact on physical health. Animals should be assessed using body condition score (BCS). HIS models typically present on the lower end of BCS 3, meaning expected appearance is closer to BCS 2+ out of 5. The animals will have a slender appearance with no excessive adipose storage and score of 2+ is considered healthy.

Animals that show signs of physical decline should be offered palliative care in the form of a low sugar supplemental gel diet (≤10% total carbohydrate), moistened feed pellets or subcutaneous injection of lactated Ringer's solution under direction of veterinary staff and reevaluated to determine if there has been improvement or decline. Progressively declining animals or animals in immediate distress should be euthanized. **Stock palliative and supportive items prior to the arrival of your cohort to allow sterility testing and ensure the supportive supplements are on hand as needed.**

Taconic uses DietGel® Recovery from Clear H2O to support the health of HIS models. DietGel® Recovery has improved hydration before and after blood draws, reduced post-procedure lethargy, and quickened recovery from alopecia. Refer to https://www.clearh2o.com/product/dietgel-recovery/ for more information.

huNOG, FcResolv® huNOG, huNOG-EXL and FcResolv® huNOG-EXL: Commonly present with mildly pilo-erected fur, poor coat condition, mild kyphosis and pale integument. This may make the mice appear hunched, scruffy and pale. Despite this presentation, the mice should still appear bright, alert, responsive and active. If the mice appear lethargic, have inflammation around the eyes and nose, have severely hunched posture or pale extremities, contact your facility's veterinary staff for evaluation.

All myeloid-supportive HIS mice have limited lifespans due to a range of outcomes including anemia, thrombocytopenia, macrophage activation syndrome and hemophagocytic lymphohistiocytosis. Lifespan varies by donor and can be impacted by environmental and experimental factors. Repeated blood sampling can negatively impact HIS mice. We recommend 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.

Watch the related Taconic webinar:

- Characterization of Myeloid Cell Hyperactivation Syndrome and Survival Differences in Humanized NOG-EXL and NSG-SGM3 Mice

huPBMC-NOG and huPBMC-B2m-NOG: NOG mice engrafted with human PBMCs will typically develop Graft vs.Host Disease (GvHD) within 4-6 weeks after engraftment. B2m-NOG mice engrafted with PBMCs have a longer useful window (8+ weeks) but will also eventually develop GvHD. GvHD manifests as weight loss, poor clinical condition, infiltration of immune cells into organs and liver damage. PBMC-engrafted NOG and B2m-NOG mice should be monitored closely for condition and be humanely euthanized when found moribund.

Certificate of Analysis

A certificate of analysis is provided on the day of shipment for all orders of HSC-engrafted mice. This certificate includes flow cytometry analysis of cell reconstitution rates collected during quality control testing (huNOG, FcResolv® huNOG, huNOG-EXL SA, and FcResolv® huNOG-EXL SA only), and a packing list correlating the tattoo ID of each animal with the data being presented.

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
Fixation Buffer	Biolegend	420801
Dimethyl Sulfoxide (DMSO)	Sigma	D2650
CountBright Absolute Counting Beads, for flow Cytometry	Thermo Fisher Scientific	C36950

Antibody information

Antibody	Clone	Fluorophore	Vendor	Catalog #
mCD45	30-F11	PerCP-Cy5.5	Biolegend	103132
hCD45	HI30	Alexa Fluor 700	Biolegend	304024
hCD19	HIB19	FITC	Biolegend	302206
hCD3	UCHT1	APC	Biolegend	300439
hCD33	P67.6	BV605	Biolegend	366612
hCD66b	G10F5	PE-Cy7	Biolegend	305116
hCD14	63D3	BV421	Biolegend	367144
hCD16	3G8	BV711	Biolegend	302044
hCD56	QA17A16	PE	Biolegend	392404
Zombie Fixable Viability Dye	N/A	Aqua	Biolegend	423101

Flow Cytometry Staining Procedures

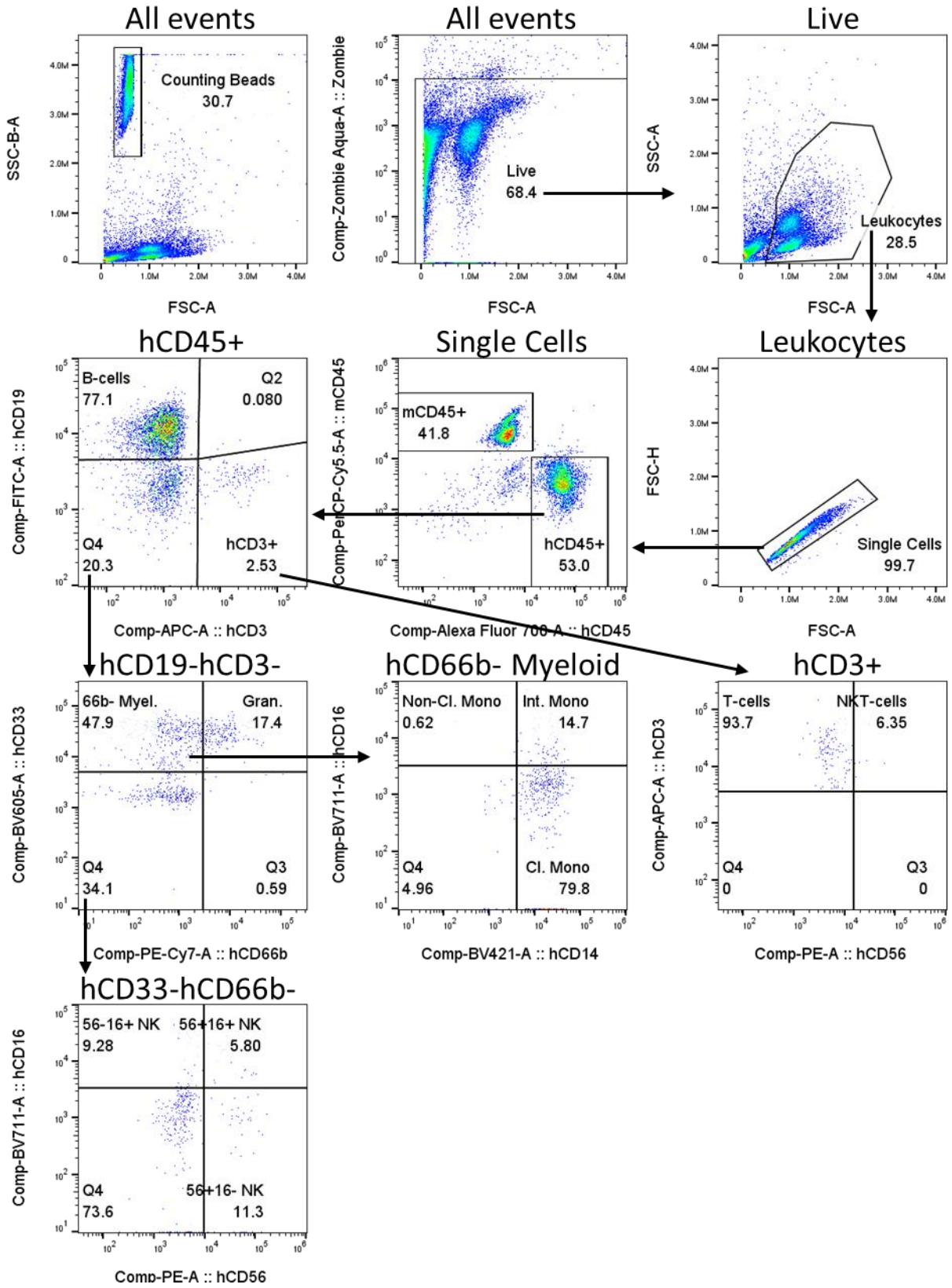
1. Add 75 µl anticoagulated whole blood (collected in K2EDTA coated tubes) to the designated well in a 96-well U bottom deep well plate
2. Add 100 µL Zombie Aqua Live/Dead Mix per sample well and gently mix (Reagent should be titrated and optimized for use in your own facility)
3. Incubate for 20 minutes at room temperature in the dark
4. After incubation, add 300 µl Wash Buffer (1X PBS with 2% FBS and 0.1% Sodium Azide) and gently mix
5. Centrifuge at 1150 RPM for 5 minutes, 4°C
6. Gently discard 200 µl of the supernatant, careful to not disturb the buffy coat
7. Add 300 µl 1X BD Lysing Solution per well and mix gently
8. Incubate for 3.5 minutes on ice
9. Centrifuge at 1150 RPM for 3 minutes, 4°C
10. Discard supernatant
11. Add 150 µl 1X BD Lysing Solution per well and mix gently
12. Incubate for 3.5 minutes on ice
13. Centrifuge at 1150 RPM for 3 minutes, 4°C
14. Do not remove supernatant
15. Add an additional 150 µl 1X BD Lysing Solution per well and mix gently
16. Incubate for 3.5 minutes on ice
17. Centrifuge at 1150 RPM for 3 minutes, 4°C
18. Discard supernatant
19. Wash cells with 150 µl standard wash buffer and gently mix
20. Centrifuge at 1150 RPM for 3 minutes, 4°C
21. Discard supernatant
22. Add 150 µl antibody mix to each well gently mix samples (Antibodies should be titrated and optimized for use in your own facility)
23. Incubate on ice for 40 min on ice in the dark
24. Add 150 µl standard wash buffer and gently mix
25. Centrifuge at 1150 RPM for 3 minutes, 4°C
26. Discard supernatant
27. Add 150 µl 1X Stabilizing Fixative or Fixation Buffer per well and gently mix
28. Incubate for 20 minutes on ice in the dark
29. Add 150 µl standard wash buffer and gently mix
30. Centrifuge at 1150 RPM for 3 minutes, 4°C
31. Discard supernatant
32. Resuspend in 400 µl standard wash buffer per well, gently mix
33. Samples may be stored overnight wrapped in aluminum foil at 4°C
34. Immediately before running samples, add 20 µL of Absolute Counting Beads to each well and mix gently

Analysis of flow cytometry data

Gating Strategy

1. Plot all events with SSC-B-A on the y-axis and FSC-A on the x-axis
    a. Gate **Counting Beads** as SSC-B-A high, FSC-A low
2. Plot all events with Zombie Aqua on the y-axis and FSC-A on the x-axis
    a. Gate **Live** cells as Zombie Aqua negative
3. Plot **Live** cells with SSC-A on the y-axis and FSC-A on the x-axis
    a. Gate **Leukocytes** including lymphocyte and granulocyte populations, while excluding the cell debris (FSC-A low, SSC-A low)
4. Plot **Leukocytes** with FSC-H on the y-axis and FSC-A on the x-axis
    a. Gate **Single Cells** along the diagonal so that FSC-H is proportional to FSC-A. Cells not on diagonal are to be excluded
5. Plot **Single Cells** with mCD45 on the y-axis and hCD45 on the x-axis
    a. Gate **hCD45+** as hCD45+, mCD45-
    b. Gate **mCD45+** as hCD45-, mCD45+
6. Plot **hCD45+** with hCD19 on the y-axis and hCD3 on the x-axis
    a. Gate **B cells** as hCD19+, hCD3-
    b. Gate **hCD3+** as hCD19-, hCD3+
    c. Gate **Q4** as hCD19-, hCD3-
7. Plot **hCD3+** with hCD3 on the y-axis and hCD56 on the x-axis
    a. Gate **T cells** as hCD3+, hCD56-
    b. Gate **NKT cells** as hCD3+, hCD56+
8. Plot **Q4 (hCD19- hCD3-)** with hCD33 on the y-axis and hCD66b on the x-axis
    a. Gate **CD66b- Myeloid cells** (66b- Myel.) as hCD33+, hCD66b-
    b. Gate **Granulocytes** (Gran.) as hCD33+, hCD66b+
    c. Gate **Myeloid cells** as hCD33+, hCD66b +/-
    d. Gate **Q4** as hCD33-, hCD66b-
9. Plot **Q1 (hCD33+, hCD66b-)** with hCD16 on the y-axis and hCD14 on the x-axis
    a. Gate **Classical Monocytes** (Cl. Mono) as hCD14+, hCD16-
    b. Gate **Intermediate Monocytes** (Int. Mono) as hCD14+, hCD16+
    c. Gate **Non-classical Monocytes** (Non-Cl. Mono) as hCD14-, hCD16+
    d. Calculate **All Monocytes** as **Classical Monocytes + Intermediate Monocytes + Non-Classical Monocytes**
10. Plot **Q4** (**hCD33-, hCD66b-)** with hCD16 on the y-axis and hCD56 on the x-axis
    a. Gate **hCD56+, hCD16- NK cells** (56+16- NK) as hCD56+, hCD16-

b. Gate **hCD56+, hCD16+ NK cells** (56+16+ NK) as hCD56+, hCD16+
c. Gate **hCD56-, hCD16+ NK cells** (56-16+ NK) as hCD56-, hCD16+
d. Calculate **All NK cells** as **hCD56+, hCD16- NK cells + hCD56+, hCD16+ NK cells + hCD56-, hCD16+ NK cells**


All events
Counting Beads
30.7
SSC-B-A
FSC-A
All events
Live
68.4
Comp-Zombie Aqua-A :: Zombie
FSC-A
Live
SSC-A
Leukocytes
28.5
FSC-A
hCD45+
B-cells
77.1
Q2
0.080
Q4
20.3
hCD3+
2.53
Comp-FITC-A :: hCD19
Comp-APC-A :: hCD3
Single Cells
mCD45+
41.8
hCD45+
53.0
Comp-PerCP-Cy5.5-A :: mCD45
Comp-Alexa Fluor 700-A :: hCD45
Leukocytes
Single Cells
99.7
FSC-H
FSC-A
hCD19-hCD3-
66b- Myel.
47.9
Gran.
17.4
Q4
34.1
Q3
0.59
Comp-BV605-A :: hCD33
Comp-PE-Cy7-A :: hCD66b
hCD66b- Myeloid
Non-Cl. Mono
0.62
Int. Mono
14.7
Q4
4.96
Cl. Mono
79.8
Comp-BV711-A :: hCD16
Comp-BV421-A :: hCD14
hCD3+
T-cells
93.7
NKT-cells
6.35
Q4
0
Q3
0
Comp-APC-A :: hCD3
Comp-PE-A :: hCD56
hCD33-hCD66b-
56-16+ NK
9.28
56+16+ NK
5.80
Q4
73.6
56+16- NK
11.3
Comp-BV711-A :: hCD16
Comp-PE-A :: hCD56

Cell population definitions

In the interest of animal welfare, blood collection for flow analysis is conducted only once. Collection occurs at 10 WPE for the huNOG-EXL SA and FcResolv® huNOG-EXL SA and at 12 WPE for the huNOG and FcResolv® huNOG. While this timepoint is sufficient for ensuring adequate peripheral blood humanization (% hCD45+ cells ≥25%), it is too early to assess peripheral reconstitution of the full spectrum of human immune cells.

Need more help?

Contact us for additional support from one of Taconic's experts.