Assaying metabolic uptake and mitochondrial function by FACS

GMD 2014-2015

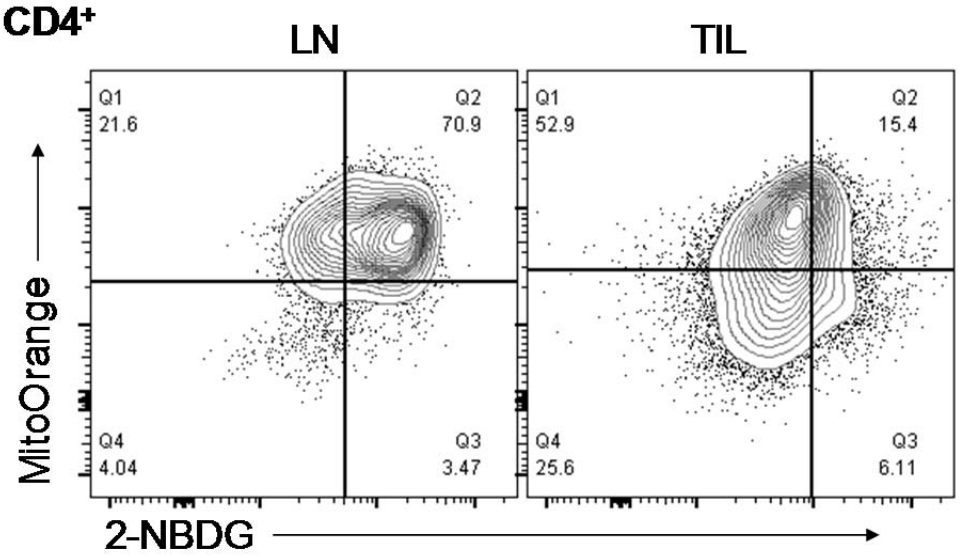
These flow cytometric assays allow for the single-cell interrogation of nutrient uptake and downstream function. Fluorescent probes allow for visualization of uptake of either glucose or free-fatty acids. This is combined with two dyes that stain total mitochondria as well as mitochondria that have a high membrane potential. Fixation and/or permeablization are not possible with these assays.

Reagents

R5 media (complete RPMI with 5% FBS)
Serum-free RPMI
FACS staining buffer
2-NBDG (Life Technologies N13195 or Cayman Chemical 11046), make up at 50mM in PBS
BODIPY FL C12 (Life Technologies D3822)
MitoTracker Orange CMTMRos (M-7510): for 1 mM solution, dilute 1 x 50 ug vial with 117 uL of anhydrous DMSO, final dilution is 1:30000
TMRE (tetramethylrhodamine ester, Life Tech): at 100 mM, 20nM final concentration
MitoStatus Red (BD Biosciences): 0.2mM solution in DMSO, final dilution is 1:20000 (10nM)
CCCP (Sigma #C2759)
MitoTracker Deep Red FM (M22426): for 1 mM solution, dilute 1 x 50 ug vial with 92 uL of anhydrous DMSO, final dilution (for T cells) is 1:100000 (10nM)
Antibodies to stain for other markers

	Channel	**Potential sensitive?**	**When to add**
TMRE	PE (bleeds heavily into PE-TR)	Yes (very)	Add to final running buffer
MitoOrange CMTMRos	PE	Yes (less so)	Stain with surface
MitoStatus Red	APC (bleeds into A700)	Yes (less so)	Stain with surface
MitoTracker Deep Red	APC (bleeds into A700)	No (total mitochondria)	Stain with surface
MitoTracker Green GM	FITC	No (total mitochondria)	Stain with surface

1. Sort, enrich, purify, or otherwise harvest tissue for analysis.
   *** This protocol uses 2-NBDG as a probe, after harvest, for 30 min *in vitro*. Alternatively, mice can be injected intravenously with 2-NBDG (100 uL of 2.5 mg/mL PBS i.v.) 30 m – 1 h prior to harvest.
2. Resuspend cells in R5 media and distribute into 96-well plates in 100 uL at no more than 1 x 10^6 cells / mL
   *** *Important controls for all assays: no probe serves as a control for the 2-NBDG/BODIPY stain, the negative control for mitochondrial membrane potential (measured with MitoOrange CMTMRos) is treatment with 100 uM CCCP, which collapses membrane potential.*
3. For **glucose uptake assays**, make a 100uM 2-NBDG solution in sfRPMI
4. For **fatty acid uptake assays**, make up a 0.5 uM BODIPY FL C12 solution in sfRPMI
   *** *Note that 2-NBDG and BODIPY fluoresce as FITC and cannot be multiplexed.*
5. Add 100 uL of the appropriate fluorescent probe-treated media and incubate at 37 degrees for 30 mins. During this time, also treat negative control wells with CCCP.
6. Wash 2 X with serum containing media.
7. Surface stain as normal, including MitoTracker dyes with your primary antibodies. For T cells, I've found that 10-30 nM final concentration works very well of positive and negative controls with minimal spectral overlap. For MitoDeepRed, ideally the dilution is in the 1:100000 range. This should be optimized for your cell type.
8. Wash cells and load into FACS tubes, if using TMRE, it should be included in your running buffer.
9. Run immediately on flow cytometer. 2-NBDG and BODIPY fluoresce in the FITC channel, Mito Orange and TMRE fluoresce in the PE channel (PE-594 is tough to compensate, but PE-Cy7 is preserved). MitoTracker Deep Red and MitoStatus fluoresce in the APC channel (Alexa 700 is tough (but possible for highly affinity antibodies) and APC-Cy7 is preserved). Doses should be optimized for your cell type and antibodies in the tandem channels.



Example data: 2-NBDG and mitochondrial staining of lymphocytes from tumor-bearing mice.