Hypoxia Green for Flow Cytometry

Catalog No. H20035

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Product information

Oxygen deficiency of the cellular environment is referred to as hypoxia, which plays a role in many diseases including lipid metabolism, inflammation, cardiovascular disease, hypertension, tumor-mediated immunosuppression, and neurodegenerative disease. Hypoxia Green reagent for Flow Cytometry is a membrane-permeant, fluorogenic probe that can help detect cells that are adapting to a low oxygen environment. As cellular oxygen levels decrease, the probe responds by releasing rhodamine, which results in detectable emissions in the green channel. This sensitive, non-antibody based probe allows for the end-point detection of hypoxic cells.

Table 1. Contents and storage

Product	Amount	Concentration	Storage*
Hypoxia Green Reagent	2 × 50 assays	30 µg/vial	• Store in freezer (–5°C to –30°C) • Dessicate • Protect from light
* When stored as directed, the product is stable for at least 6 months from the date receipt. **Approximate Ex/Em maxima:** 505 nm/524 nm			

Materials required, but not provided

- Cells of interest
- DMSO, Anhydrous (Cat. No. D12345)
- Phosphate-buffered saline (PBS) or similar protein-free buffer
- Culture media containing protein such as FBS or BSA
- Flow cytometer

Caution

No data are available addressing the mutagenicity or toxicity of the Hypoxia Green reagent. Handle the DMSO with caution because DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

Storage and handling

Upon receipt, store the kit components desiccated at ≤–20°C until required for use. When stored properly DMSO and dry Hypoxia Green reagents are stable for at least 6 months. Allow the products to warm to room temperature before opening the vials. Stock solution can be frozen after use, but should be aliquoted to avoid repeated freezing and thawing.

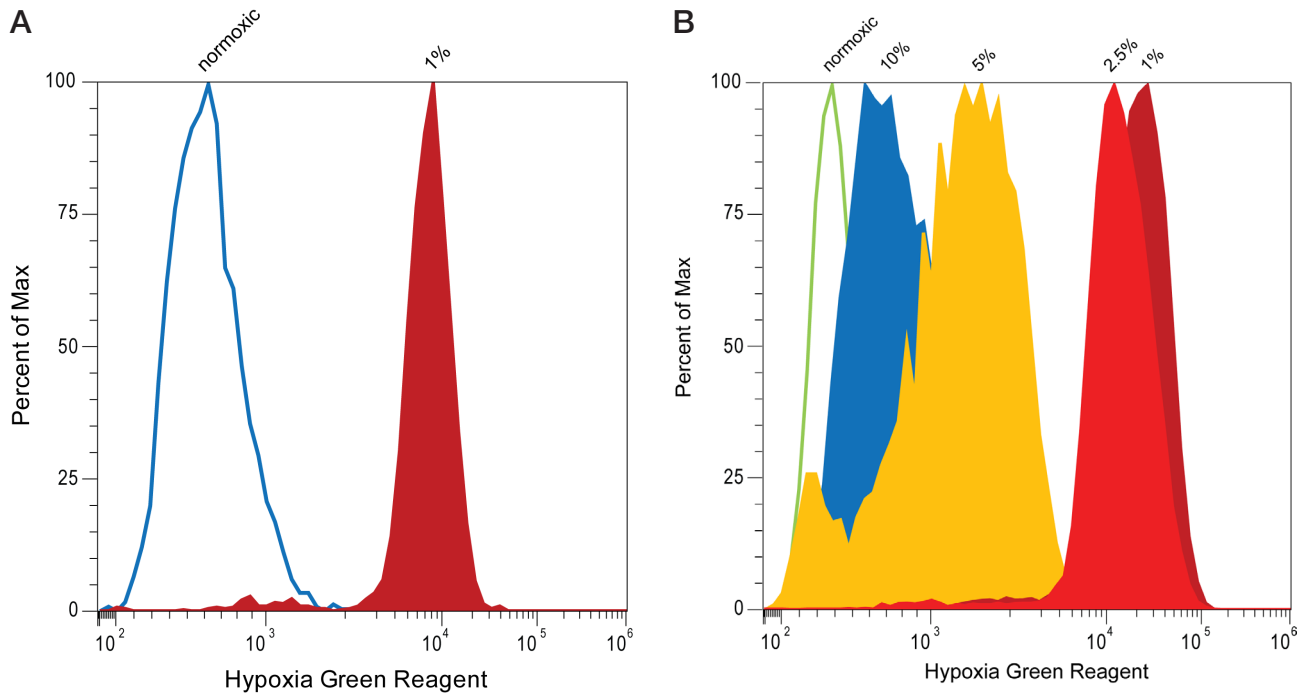

Figure 1. Detection of hypoxic cells using Hypoxia Green Reagent for Flow Cytometry. Jurkat cells, a human leukemia cell line, were incubated under normoxic or hypoxic conditions for 18 hours. Hypoxia Green reagent was subsequently added to the cells and the cells were incubated for an additional 3 hours. Cells were harvested and analyzed on an Attune™ NxT Flow Cytometer using a 488-nm laser and a 530/30-nm emission filter. Normoxic cells (subjected to 20% O_2) can be distinguished from **(A)** hypoxic cells (subjected to 1% O_2, red histogram peak) as well as **(B)** hypoxic cells subjected to different O_2 concentrations (10% O_2 blue peak; 5% O_2 yellow peak; 2.5% O_2 orange peak and 1% O_2 red peak).

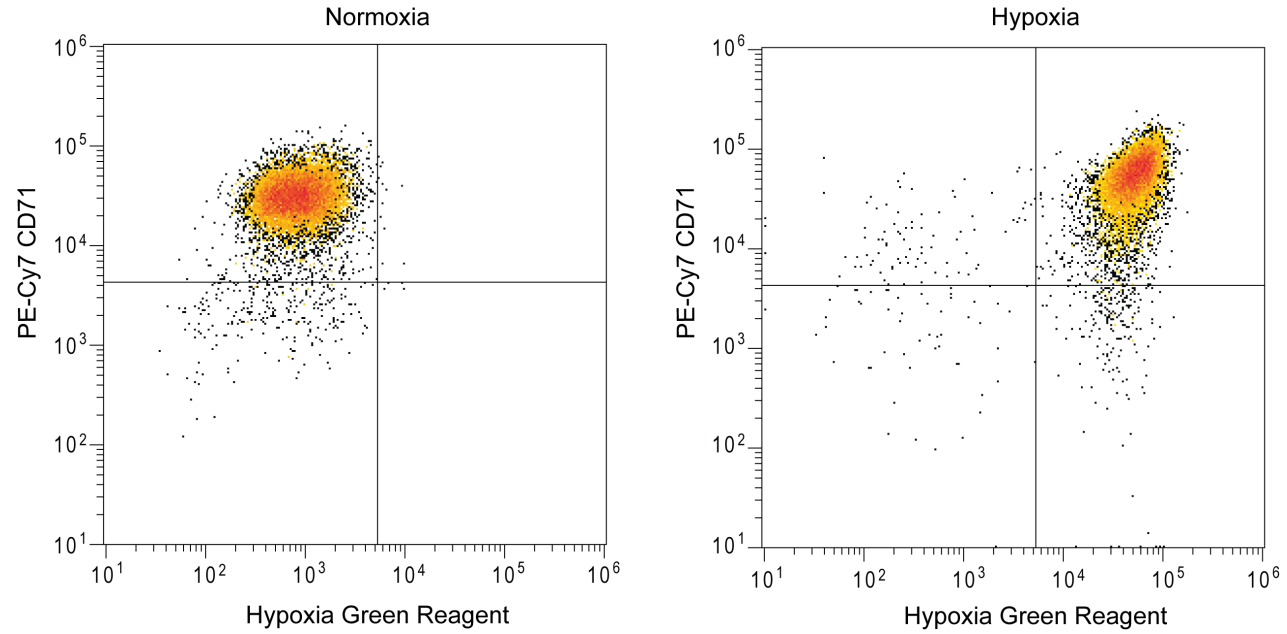

Figure 2. Simultaneous detection of hypoxia and a cell surface marker in K562 leukemia cells. K562 cells, a human leukemia cell line that expresses CD71 (transferrin receptor), were incubated under normoxic (20% O_2) or hypoxic (1% O_2) conditions for 18 hours. Hypoxia Green reagent was subsequently added to the cells and the cells were incubated for an additional 3 hours. Cells were harvested, stained with PE-Cy7 anti-CD71 antibody, then analyzed on an Attune™ NxT Flow Cytometer using a 488-nm laser and a 530/30-nm emission filter to detect Hypoxia Green, and a 561-nm laser and a 780/60-nm emission filter to detect CD71.

Methods

Prepare stock solution

Resuspend the Hypoxia Green reagent in 50 µL of DMSO to create a 1 mM stock solution.

Stain cultured cells with Hypoxia Green reagent

1. Culture the cells according to your protocol.
2. Add 1 µL of the 1 mM Hypoxia Green reagent to 1 mL of 1×10^6 cells.

   Note: Staining can be performed on cells in culture media.
3. Incubate the cells at 37°C for 2–3 hours.
4. *Optional*: Wash the cells once in PBS.
5. Proceed with analysis.

Ordering information

Cat. No.	Product name	Unit size
H20035	Hypoxia Green for Flow Cytometry	2 × 50 tests
Related products		
D12345	DMSO, Anhydrous	10 × 3 mL

Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision	Date	Description
A.0	16 February 2018	New user guide

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