Protocols for oxygen scavenging systems

Buffers
Protocol
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To prevent damage to (short) DNA tethers or bleaching of fluorophores by reactive oxygen species, it is recommended to use an oxygen scavenging system. Here we describe two systems, one that couples the Glucose Oxidase and Catalase (GODCAT) in a buffer containing glucose and the other that employs Trolox. While the two systems can be used separately, a combination of both systems is recommended for optimal results.

1. Glucose Oxidase and catalase system (GODCAT)

The following products are recommended:

- Glucose Oxidase, Aspergillus niger, Recombinant (Sigma-Aldrich, 345386-10KU, 250 units/mg).
- Catalase from bovine liver (Sigma Aldrich, C100-50MG, 40,000-60,000 units/mg) .
- D-(+)- Glucose (Sigma Aldrich, G8270).
- Storage buffer: 40 mM Tris pH 7.5, 140 mM NaCl, 0.1 mM EDTA. It is recommended to use 40–60 mM Phosphate or Tris buffer at pH 7.5 with 20–150 mM common salts (e.g., KCl or NaCl).
- 0.22 µm filter.

Preparation of the stock solution

Glucose Oxidase (final concentration: 100x, 16,500 units/ml):

- Check the units/mg of Glucose Oxidase of the batch that you are using. The units/mg of Glucose Oxidase may vary from batch to batch.
- Prepare a 16,500 units/ml solution of Glucose Oxidase with storage buffer.
- Vortex the solution.
- Filter the solution with a 0.22 µm filter.

Catalase (final concentration: 100x, 217,000 units/ml):

Note: Handle the Catalase according to instructions from the manufacturer. Prevent exposure of the stock to air.

- Check the units/ml of Catalase of the batch that you are using. The units/ml of Catalase may vary from batch to batch.
- Prepare a 217,000 units/ml solution of Catalase with storage buffer.
- Vortex the solution.
- Filter the solution with a 0.22 µm filter.

Mixing and aliquoting:

- Mix the 100x Glucose Oxidase and 100x Catalase in a ratio 1:1 to arrive at a 50x working stock. Snap freeze and store at -80 °C.

Preparation of GODCAT oxygen scavenging system

Prepare buffer of choice containing 1x Glucose Oxidase-Catalase mix and 0.65% glucose.

The GODCAT oxygen scavenging system acidifies the sample over time. Therefore, it is recommended to change the buffer every two hours.

2. Trolox buffer solution

The following products are recommended:

- Trolox® 97% (Thermo Scientific™ Acros, 218940050)
- 10 M NaOH solution
- 0.22 µm filter

Preparation of the Trolox stock solution

The protocol below is for the preparation of 50 ml.

- Add 65 mg of Trolox to 50 ml water
- Add 25 µl of 10 M NaOH to the solution
- Incubate the Trolox solution for 20-24 h at room temperature while rotating to ensure constant mixing. This will result in a saturated ~2 mM solution of Trolox at pH 4.5.
- Filter the ~2 mM saturated solution of Trolox with a 0.22 µm filter and store at + 4 °C.

The filtered Trolox can be stored up to two weeks at +4 °C.

Preparation of Trolox buffer

Prepare the buffer of choice supplemented with Trolox at ≥ 1 mM concentration. A 40-60 mM concentration of buffering agents such as Phospate or Tris-HCl (pH 7.5-8) is recommended in your buffer of choice to balance the lower pH of Trolox solution and obtain a final pH ranging between 7 and 8. It is recommended to check the pH after supplementing the buffer of choice with Trolox.

3. Preparation of GODCAT - Trolox scavenging system

For optimal results, an oxygen scavenging system made by a mix of Trolox and GODCAT is recommended. Supplement buffer of choice with Trolox at ≥ 1 mM concentration, 0.65% glucose and 1x Glucose Oxidase-Catalase mix.

The presence of GODCAT acidifies the sample over time. Therefore, it is recommended to use fresh oxygen scavenging system every two hours

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