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Measurement of glucose in cell culture media via the ABTS-linked glucose oxidase-peroxidase assay

Reagents

Sigma A1888 ABTS; [2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)- diammonium salt]

Sigma P6782 Type VI-A, essentially salt-free, lyophilized powder, ~1000 units/mg solid (using ABTS), 250-330 units/mg solid (using pyrogallol) ; pH optimum for ABTS = 5.0, 25^{o}C

Sigma G7141 Glucose Oxidase from *Aspergillus niger*. Type X-S, lyophilized powder, 100,000-250,000 units/g solid;
pH optimum = 5.5, 35^{o}C

Stock solutions

1. ABTS = 50 mg/mL in H20. (4^{o}C, >2 months)

2. HRP (PERID) 10x = 5.0 mg/mL in 50/50 0.2M Na-phosphate/glycerol (-20^{o}C, >1yr)

3. Glucose Oxidase (GOD) 10x = 20mg/mL in 50/50 0.2M Na-phosphate/glycerol (-20^{o}C, >1yr)

4. 1M Na-phosphate, pH 6.3-6.5@RT = 50/50 1M Na-phosphate Monobasic/1M Na-phosphate Dibasic

5. 2M NaAc @pH 4.6@RT = 50/50 2M NaAc/2M AcAc (dilute 10x to 0.2M for assay)

6. 0.5N NaOH

7. Glucose standards in H20, top standard @10ug/uL --> 0.3125ug/uL

Note: Make 1x stocks of HRP and GOD in 50/50 0.2M Na-phosphate/glycerol.

Master mixes

GOD-PERID rxn mix, per sample:

H20:	73uL
1M Na-phosphate, pH 6.3-6.5@RT:	20uL
Add 93uL of GOD-PERID buffer +	3uL of ABTS, 50mg/mL
	2uL of **1x** HRP
	2uL of **1x** GOD

100uL final volume = sufficient for 1 sample (enough substrate and enzyme to max out absorbance (>10ug glucose oxidizing capacity)

1. Determine the volume of media you can measure. Keep in mind that the upper limit of this assay is approximately 7.5-10ug of glucose/well, depending on how quickly you read the absorbance. The following table is a quick reference to determine ug of glucose per uL of media at common glucose concentrations:

Molarity	ug/uL
1.0	0.18
2.5	0.45
5.0	0.90
7.5	1.35
10.0	1.80
15.0	2.70
20.0	3.60
25.0	4.50

2. When measuring typical culture media that contains 5-25mM glucose and/or serum, BSA, phenol red, or other potentially interfering factors, you must dilute the media to minimize interference/quenching of the assay. The recommended buffer is:

100uL 0.2M NaAc @pH 4.6 (RT)
10uL 0.5N NaOH

Add the desired volume of media to this buffer. To save time, you may perform this step directly in a 96-well plate. Otherwise, use microfuge tubes.

3. Make your glucose standard curve in the same manner as you prepared your samples. Add 1uL of standard for each point. To be technically correct, you should also add 1uL of H20 to each sample.

4. Add 100uL of the GOD-PERID rxn mix to each sample and to your standards. A repeater pipette is strongly recommended to minimize time bias.

5. Incubate at RT for at least two minutes. The assay is stable for 10-60 minutes, depending on the volume of media and any quenching agents that may be present. It may be necessary to run several standard curve tests to determine optimal media volume and incubation time. In the absence of quenching agents, under ideal conditions, the assay is perfectly linear, with a lower limit of approximately 0.2ug of glucose and an upper limit of ~7.5ug glucose.

Notes:

A. This assay has been tested with up to 200uL of glucose-free, phenol red-free, serum-free DMEM. There is some quenching effect, but the assay is stable and linear for up to 10 minutes under this condition.