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Ethanol Quantification assay

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Protocol status: Working
We use this protocol and it's working

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Abstract

Chemoenzymatic method for quantification of ethanol with a spectrophotometer at 500 nm (not UV !)

Based on **Lewicka 2014** (DOI: 10.1021/sb500020g)

Adapted for analysis in plate reader of multiple samples at the same time.

Materials

Yeast Alcohol Dehydrogenase - Sigma...
NAD+ - Sigma
Phenazine methosulfate (PMS) - Sigma
Iodonitro-tetrazolium violet (INTV) -Sigma

50 mM Tris-HCl buffer (pH 7.5)
50 mM tris-HCl pH= 7.5 (0.118g tris base + 0.635g tris.HCl in 100 ml of H2O)
(or buy pre-made tris pH 7.5 and dilute it accordingly)

Ethanol absolute to be used as standard.

Multichannel pipette
Flat-bottom 96-well plate

Prepare culture samples

1 Sample your culture and spin it 16000 rpm, 00:01:00 .

Transfer Supernatant to new tube and keep On ice if analysed inmediately, or freeze if analysed in a different day.

Prepare stocks

2 Prepare the following stocks using your tris buffer:

	Stock	mg/mL	mM	Mw
	Yeast Adh	**5**		
	NAD+	**13.3**	**20**	685.41
	PMS	**6.1**	**20**	306.34
	INTV	**25.3**	**50**	505.00
	Ethanol	46 (36.3 uL/mL)	1000	46

INTV needs to be dissolved in buffer-DMSO (50:50)

Keep reagents On ice
(no need for ethanol)

Keep the buffer at Room temperature !

3 Prepare your ethanol standard ladder by making different dilutions of your 1M ethanol with tris buffer. The standard could be, for example, 400-200-100-50-25-10-5-0 mM ethanol.

Prepare reaction mastermix

4 Prepare reaction mix for all your samples.

Calculate the volume to be prepared should be **200 µL * well * 1.1** (so you have)
When counting "wells", consider replicates for samples and standards.

That is the volum of Tris you need, to which you have to add:

- Yeast ADH: 5 µL per mL
- NAD+: 1µL per mL
- PMS: 1 µL per mL
- INTV: 20 µL per mL

Reaction plate and incubation

5 Plan how to load samples in plate, and annotate it.

Consider that the reaction mix should be loaded to the replicates at different moments (so their variability comprises the variability caused by loading the mix at the different times.)

5.1 Load 200 µL of buffer to the blank wells. (some replicates here are good too). These wells wont receive reaction mix.

5.2 Load 10 µL of sample to the well bottom. Be sure all the volume of liquid is in the well and not in your pipette tip.

5.3 Load 200 µL of reaction mix to the wells with your multichannel pipette with as little difference of time between sample as possible.

6 Mix & incubate

6.1 Mix the plate (the plate reader can do this).

6.2 Incubate the plate for Room temperature for 15 minutes, **in the dark.**

7 Measure Abs 500 nm in plate reader at Room temperature

Note

Changes in temperature can cause condensation in the plate lead, which will affect readings.

Analysis and troubleshooting

8 Blank should be done with water, samples measurements will be reliable only in the linear region of the standard.

BMG plate readers do most of the analysis for you, if you tell the software which wells had each blank, standarda and sample. You can introduce this in the software before or after the readings.

You can have a positive control (negative control culture spiked with ethanol).

Sensitivity can be increased using 20 µL of sample in 200 µL of mastermix, or increasing incubation time to 30 minutes.

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