[protocol] BCA method

woensdag 13 mei 2015 11:05

New:	Old:
bca1bul	BCA method
BCA checklist	

Kit is in sample fridge

- [ ] **Make sure the samples are transparent (filter if needed with syringe filter 0.2 µm)**
  Estimate the calibration curve range: e.g. 0.1% max for Secondary emulsions nonadsorbed? = 1 g/L max
- [ ] Make sure you have calibration samples ready (fresh or defrozen):
  Stock: 10 g/L Citric acid buffer (10 mM; pH 3)

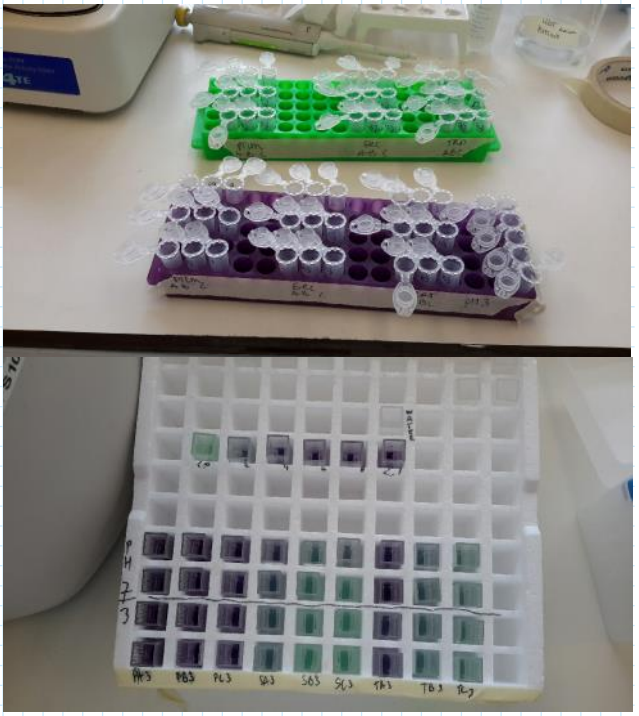
Conc (g/L)	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
Vstock (uL)	*0*	*20*	*40*	*60*	*80*	*100*
Vbuffer (uL)	*1000*	*980*	*960*	*940*	*920*	*900*
Vtotal	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL

Ór Stock: 1.0 ml of a standard solution consisting of 1.0 g/L BSA in 0.15 M NaCl with 0.05% sodium azide as a preservative.. Lower volume calibration curve:

Conc (g/L)	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1**
Vstock (uL)	*0*	*0.2*300=60*	*0.4*300=120*	*0.6*300=180*	*0.8*300=240*	*300*
Vbuffer (uL)	*400*	*340*	*280*	*220*	*160*	*0*
Vtotal	0.2 mL					

60+120+180+240+300=900 mL
DEZE EPJES INVRIEZEN!!!

- [ ] Prepare fresh bca working reagent:
  50 parts of A plus 1 part B, shelf life of a day
  so for 12*3+6=42 tubes you need for example 50 ml to be sure: 50ml A plus 45/50=1 ml B
- [ ] Take 50ul from the calibration solutions (0 tot 1 g/L calibration curve; 50 uL sample; als je verwacht dat je sample hogere concentratie heeft moet je hem vooraf verdunnen! Niet minder toevoegen)
  Can be frozen in between.
- [ ] Dilute "samples" 5x and 10x in buffer: 100 uL in 400 / 900 uL buffer respectievelijk, vortex!
- [ ] Take twice 50ul of the undiluted and diluted samples (A, B, C) and add them to another eppendorf tube
- [ ] Add 1ml working reagent to all these epjes (A, B, C, calibration curves).
- [ ] Incubate 2h at room temperature, ór 30 min at 37°C in shaking device
- [ ] Cool to fridge 5min, then 10 min room
- [ ] Measure absorbance at Fixed wavelength at 562 nm: in 1ml cuvettes
  - [ ] **Blanc is the zero concentration, measure this first**
  - [ ] Start timer and measure calibration curve
  - [ ] Measure samples
  - [ ] Measure calibration curve
  - [ ] Write down total time of measuring: min (should be <10 min!)
- [ ] Turn off spectrophotometer
- [ ] Dispose the reagent in the sink with a lot of rinsing



Concentration (g/L)	absorbance at 562 nm
0	
0.2	
0.4	
0.6	
0.8	
1	

Primary emulsion	Abs 562 nm	duplo
Sample before (pH3) A		
Sample before (pH3) B		
Sample before (pH3) C		
Sample after (pH7) A		
Sample after (pH7) B		
Sample after (pH7) C		

Secondary	Abs 562 nm	duplo
Sample before (pH3) A		
Sample before (pH3) B		
Sample before (pH3) C		
Sample after (pH7) A		
Sample after (pH7) B		
Sample after (pH7) C		

Tertiary emulsion	Abs 562 nm	duplo
Sample before (pH3) A		
Sample before (pH3) B		
Sample before (pH3) C		

Sample after (pH7) A		
Sample after (pH7) B		
Sample after (pH7) C		

Concentration (g/L)	absorbance at 562 nm Duplo at the end
0	
0.2	
0.4	
0.6	
0.8	
1	