pH tolerance assay for Vibrio natriegens

Daniel Marchal

Abstract

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Before start

Media preparation:

1M KH_2PO_4 100ml → 13,61g

1M K_2HPO_4 100ml → 17,42g

1M Na-Acetate 100ml → 13,61g Trihydrate

1M Acetic acid 100ml → 60,05g (liquid)

1M Glycine 100ml → 7,51g

Autoclave KH_2PO_4, K_2HPO_4 and Na-Acetate and sterilfiltrate Glycine, Acetic acid should be sterile yet

LB2,5:

25g/l LB broth (Miller)

15g/l NaCl

BHIv2:

37g/l Brain heart infusion broth

204 mM NaCl

4.2 mM KCl

23.14 mM $MgCl_2$

Materials

- Sodium acetate View by P212121
- Potassium phosphate (dibasic) View by P212121
- Lysogeny broth by Contributed by users
- Hydrochloric Acid by Contributed by users
- brain Heart Infusion Broth Oxoid CM1135-UK by Contributed by users
- Sodium hydroxide SB0617.SIZE.500G by Bio Basic Inc.
- Monopotassium phosphate by Contributed by users
- Acetic acid, glacial 1.01830.2500 by Merck Millipore
- Glycine 50046 by Sigma

Protocol

Step 1.

Inoculate precultur of BHIv2 with *V. natriegens* and incubate oN at 30°C

Step 2.

Prepare buffer solutions for each pH value in the table.

All stock solutions should be at the same molarity (volumens refer to an buffer volume of 50ml):

pH	Acid	V(Acid-Stock)	Base	V(Base-Stock)
2.4	HCl	32,40	Glycine	50,00
3	HCl	11,40	Glycine	50,00
3.5	Acetic acid	2,60	Sodium acetate	47,40
4	Acetic acid	7,40	Sodium acetate	42,60
4.5	Acetic acid	17,73	Sodium acetate	32,27
5	Acetic acid	31,74	Sodium acetate	18,26
5.5	Acetic acid	42,30	Sodium acetate	7,70
6	KH_2PO_4	2,90	K_2HPO_4	47,10
6.5	KH_2PO_4	8,16	K_2HPO_4	41,84
7	KH_2PO_4	19,07	K_2HPO_4	30,93
7.5	KH_2PO_4	33,05	K_2HPO_4	16,95
8	KH_2PO_4	43,02	K_2HPO_4	6,98
8.5	Glycine	50,00	NaOH	4,00
9	Glycine	50,00	NaOH	8,80

9.5	Glycine	50,00	NaOH	22,40

Step 3.

Measure pH and adjust with HCl/NaOH

Step 4.
Prepare 5ml flasks with 4ml BHI + 1mlbuffer (see table) for pH2 – pH9

Step 5.

Measure pH and adjust with HCl/NaOH

Step 6.

Sterilfiltrate into a new tube (because the medium is contaminated by the pH meter)

Step 7.
Inoculate flasks with 10µl preculture

Step 8.
Mix well and incubate at 37°C shaking

Step 9.

Take samples after 15min and 6h and make 1:10, 1:100 and 1:1000 dilutions

Step 10.

Plate out 50µl of each dilution on LB2,5 medium

Step 11.
Incubate plates at 37°C oN and determine CFUs