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Modified pH drop protocol (K⁺)

Elizabeth Fozo¹¹In-house protocol

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Works for me

This protocol is published without a DOI.



Eadewunm

ABSTRACT

Modified pH drop protocol (K⁺)

PROTOCOL CITATION

Elizabeth Fozo 2020. Modified pH drop protocol (K⁺). **protocols.io**
<https://protocols.io/view/modified-ph-drop-protocol-k-bqksmuwe>

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GUIDELINES

- Prepare the K⁺ electrode for use
- Do slope check for electrode performance
- Standard curve
- Modified pH drop protocol

MATERIALS TEXT

K⁺ electrodes are made from the following solutions:

- 1M KCl to dilute for standard curve
- 1M NaCl to make the 0.1M background solution
- 1mM MgCl₂ as the “neutral salts” solution for the modified pH drop protocol (noKCl)
- 0.5M NaOH to pH the cell suspension to 7.2 for the modified pH drop protocol (noKCl)
- 0.1M KCl to add to cells with glucose for modified protocol

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ABSTRACT

Modified pH drop protocol (K^+)

BEFORE STARTING

Here are some notes from my notebook from 2002:

- When using the K^+ electrode, need to read mV for the standard curve.
- Also need to do ALL readings in a 0.1M NaCl background.
- These electrodes only have a 6-month life span.

To prepare the K^+ electrode for use:

- 1 Remove the rubber caps.
- 2 Fill with 0.1M NaCl filling solution provided to just below the hole in the probe.
- 3 Gently shake the probe like a thermometer to remove all air bubbles.
- 4 Immerse the electrode in 0.1M KCl for 20-30 minutes.
- 5 Rinse off.

Do slope check for electrode performance:

- 6 1. Put 0.1M NaCl in a beaker. Stir. Put the meter at the mV setting.
- 7 Lower the K^+ electrode into the beaker.
- 8 Add 1mL 0.1M KCl to the beaker & keep stirring. Once the reading is stable, record the mV reading (mine was -49.2).
- 9 Add 10mL 0.1M KCl to the beaker. Continue stirring. Record mV reading (mine was 6.1).
- 10 The difference between the readings should be $52\text{mV} \pm 2$. (Mine was 55.3mV – called that good enough!)

Standard curve:

- 11
 - 0mM (no K⁺) = 9mL water + 1mL 1M NaCl
 -
- 12 Take 1M stock KCl & dilute 1:10 in 8mL water + 1mL 1M NaCl to create the following K⁺ concentrations:
 - 0.1M (10⁻¹)
 - 0.01M (10⁻²)
 - 1mM (10⁻³)
 - 0.1mM (10⁻⁴)
 - 0.01mM (10⁻⁵),
 - 1μM (10⁻⁶)
- 13 Read mV for each standard. Create standard curve: electrode potential (mV) vs. K⁺ concentration (mM)

Modified pH drop protocol:

- 14 Prepare 100mL ON of culture as per pH drop protocol (in BHI + 1% glucose).
- 15 Measure OD₇₀₀.
- 16 Pellet cells.
- 17 Wash cell pellet 2X in 30-35mL 1mM MgCl₂.
- 18 Resuspend pellet in x mL 1mM MgCl₂, where x = # from formula
- 19 For each experiment, take 4.5 mL cells + 0.5mL 1M NaCl → 0.1M NaCl in the final 5mL cell suspension.
- 20 Adjust pH to 7.2 with 0.5M NaOH.
- 21 Add 100μL 50% glucose = time zero.
- 22 Read pH & mV readings at desired intervals.