Electronic Supplementary Information (ESI) for

Quantification of α-Polylysine: A Comparison of Four UV/Vis Spectrophotometric Methods

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**BCA assay Kit from Sigma-Aldrich (BCA)**\(^1\)

**Bicinchoninic acid (BCA) solution**

Product B9643 from Sigma-Aldrich

Contains bicinchoninic acid (BCA), sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1 N NaOH (final pH = 11.25). The BCA-solution is made as follows (2): 0.1 g sodium bicinehoninate, 2.0 g Na\(_2\)CO\(_3\)·H\(_2\)O, 0.16 g sodium tartrate dehydrate, 0.4 g NaOH, 0.95 g NaHCO\(_3\), made up to 100 mL with deionized water. If necessary, adjust the pH to 11.25 with NaHCO\(_3\) or NaOH.\(^2\)

**M (BCA disodium salt) = 388.27 g/mol**

**CAS number of BCA: 1245-13-2**

**Copper(II) sulfate pentahydrate (CuSO\(_4\) · 5 H\(_2\)O) solution**

4% (w/v) solution prepared in water

Product C2284 from Sigma-Aldrich

**M (CuSO\(_4\)·5 H\(_2\)O) = 249.69 g/mol**

**CAS number of CuSO\(_4\)·5 H\(_2\)O: 7758-99-8**

**Protein Standard Bovine Serum Albumin (BSA) solution**

Product P0914-5AMP from Sigma-Aldrich

5 flame-sealed glass ampoules, each containing 1.0 mL of a solution containing 1.0 mg BSA in 0.15 M NaCl and 0.05% NaN\(_3\)

**References cited**

\(^1\) www.sigmaaldrich.com (accessed February 2010)

Protocol 1. Trypan Blue (TB) Assay

Working solutions:

- **MES buffer solution**: 0.1 M MES, 0.15 M NaCl, pH 4.7
- **α-PDL stock solution**: 2 mg/mL α-poly-D-lysine hydrobromide (mol wt 15,000–30,000), prepared in MES buffer solution.
- **α-PDL analyte solution**: Prepared from the α-PDL stock solution by dilution with deionized water to a final MES concentration of 0.01 M and a final NaCl concentration of 0.015 M. The polylysine analyte solution contains between 1 and 9 μg/mL α-poly-D-lysine hydrobromide.
- **Reagent A**: 1 mg/mL trypan blue in deionized water

Procedure Standard assay:

1. Add 50 μL Reagent A to 1250 μL α-PDL analyte solution and mix well.
2. Incubate the mixture at 37 °C for 1 h.
3. Cool to room temperature and centrifuge at 8,000 rpm for 20 min.
4. Remove the supernatant and record at room temperature the absorption spectrum of the supernatant against MES buffer solution between 200 and 800 nm using a 1.5 mL, 1 cm quartz cell (Figure 1(a)).
5. Plot the absorbance measured at 580 nm against the concentration of α-poly-D-lysine hydrobromide in the α-PDL analyte solution (Figure 1(b)).

Procedure Micro assay:

6. Add 5 μL Reagent A to 125 μL α-PDL analyte solution and mix well.
7. Incubate the mixture at 37 °C for 1 h.
8. Cool to room temperature and centrifuge at 8,000 rpm for 20 min.
9. Remove the supernatant and record at room temperature the absorption spectrum of the supernatant against MES buffer solution between 200 and 800 nm using a 100 μL, 1 cm quartz cell.
10. Plot the absorbance measured at 580 nm against the concentration of α-poly-D-lysine hydrobromide in the α-PDL analyte solution.
Reaction conditions (step 2 of the Procedure) and concentrations in the sample cell:

\[ [TB] = 40 \, \mu M, \text{pH 4.7, } 37 \,^{\circ}\text{C, 60 min reaction time.} \]

Concentration of lysine residues: 4.6 – 41.4 \, \mu M.

Approximate volumes required for 50 measurements:

- 0.5 mL 0.1 M MES buffer solution, containing 0.15 M NaCl, pH 4.7
- 0.5 mL \( \alpha \)-PDL stock solution (2 mg/mL MES buffer solution)
- 65 mL deionized water
- 2.5 mL Reagent A (1 mg/mL TB in deionized water)
Protocol 2. TNBS Assay

**Working solutions:**

- **MES buffer solution:** 0.1 M MES, 0.15 M NaCl, pH 4.7
- **α-PDL stock solution:** 2 mg/mL α-PDL hydrobromide (mol wt 15,000 – 30,000), prepared in MES buffer solution.
- **α-PDL analyte solution:** Prepared from the α-PDL stock solution by dilution with MES buffer solution. The polylysine analyte solution contains between 10 and 60 μg/mL α-PDL hydrobromide.
- **Reagent A:** 5 w/v % 2,4,6-trinitrobenzene sulfonic acid (TNBS) in methanol
- **Reagent B:** 0.1 M borate buffer, pH 8.5
- **Reagent C (freshly prepared):** Mixture of Reagent A and Reagent B in a ratio of 1 to 200 (v/v)
- **Reagent D:** 1 N NaOH
- **Reagent E:** 10 wt% sodium dodecylsulfate (SDS) in deionized water
- **Reagent F:** 1 N HCl

**Procedure:**

1. Add 250 μL Reagent C to 500 μL α-PDL analyte solution and mix well.
2. Add 50 μL Reagent D and mix well.
3. Incubate the mixture at 37 °C for 30 min.
4. Add 250 μL Reagent E and mix thoroughly but carefully (avoid foaming!).
5. Add 125 μl Reagent F and mix carefully (avoid foaming!).
6. Record at room temperature the absorption spectrum against Reagent B between 250 and 600 nm using a 1.5 mL, 1 cm cell (Figure 2(a)).
7. Plot the difference in absorbance measured at 344 nm in presence and absence of polylysine against the concentration of α-PDL hydrobromide in the α-PDL analyte solution (Figure 2(b)).

**Reaction conditions (step 3 of the Procedure) and concentrations in the sample cell:**

[TNBS] = 265 μM, pH 9.0 – 9.5, 37 °C, 30 min reaction time.
Concentration of lysine residues: 20.4 – 122.6 μM.
Approximate volumes required for 50 measurements:

30 mL 0.1 M MES buffer solution, containing 0.15 M NaCl, pH 4.7
1.0 mL Polylysine stock solution (2 mg/mL MES buffer solution)
65 mL deionized water
0.1 mL Reagent A (5 w/v % TNBS in methanol)
13 mL Reagent B (0.1 M borate buffer, pH 8.5)
13 mL Reagent C (1:200 (v/v) mixture of Reagent A and Reagent B)
3 mL Reagent D (1 N NaOH)
13 mL Reagent E (10 wt % SDS)
7 mL Reagent F (1 N HCl)
Protocol 3. OPA Assay

Working solutions:

- **PBS (phosphate buffered saline) solution**: 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2.
- **α-PDL stock solution**: 2 mg/mL α-poly-D-Lys hydrobromide (mol wt 15,000 – 30,000), prepared in PBS solution.
- **α-PDL analyte solution**: Prepared from the α-PDL stock solution by dilution with PBS solution. The polylysine analyte solution contains between 20 and 670 μg/mL α-PDL hydrobromide.
- **Reagent A**: 40 mg/mL OPA (o-phthalaldehyde) in ethanol.
- **Reagent B**: 0.4 M borate buffer, pH 9.5.
- **Reagent C**: 20 wt% sodium dodecylsulfate (SDS) in deionized water.
- **Reagent D**: 2-mercaptoethanol
- **Reagent E (freshly prepared)**: Mix 1 mL Reagent A, 25 mL Reagent B, 12.5 mL Reagent C and 0.1 mL Reagent D. In a measuring flask add deionized water to yield a total volume of 50 mL.

Procedure:

1. Put two 1.5 mL, 1 cm cells, a sample cell and a reference cell, into the double beam spectrophotometer and fill both cells with 1 mL Reagent E.
2. Add 25 μL α-PDL analyte solution to the sample cell, shake gently (avoid foaming!) and follow the increase in absorbance at 337 nm for up to 300 s at room temperature (Figure 3(b)).
3. Plot the absorbance measured at 337 nm after 300 s against the concentration of α-PDL hydrobromide in the α-PDL analyte solution (Figure 3(c)).

Reaction conditions (step 2 of the Procedure) and concentrations in the sample cell:

[OPA]=5.8 mM, [2-mercaptoethanol]=27.8 mM, [SDS]=173 mM, pH 9.5, 22 °C, 5 min reaction time.

Concentration of lysine residues: 2.3 – 78 μM.
Approximate volumes required for 50 measurements:

1.0 mL PBS (0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2)
0.5 mL α-PDL stock solution (2 mg/mL MES buffer solution)
1 mL Reagent A (40 mg/mL OPA in ethanol)
25 mL Reagent B (0.4 M borate buffer, pH 9.5)
12.5 mL Reagent C (20 wt % SDS in deionized water)
0.1 mL Reagent D (2-mercaptoethanol)
10 mL deionized water
50 mL Reagent E (mixture of Reagent A, B, C, and D and deionized water)
Protocol 4. BCA Assay

**Working solutions:**

- **MES buffer solution:** 0.1 M MES, 0.15 M NaCl, pH 4.7.
- **α-PDL stock solution:** 2 mg/mL α-PDL hydrobromide (mol wt 15,000 – 30,000), prepared in MES buffer solution.
- **α-PDL analyte solution:** Prepared from the α-PDL stock solution by dilution with MES buffer. The polylysine analyte solution contains between 200 and 1000 μg/mL α-PDL hydrobromide.
- **Reagent A:** Bicinchoninic acid solution B9643 from Sigma-Aldrich (contains 2.57 mM BCA).
- **Reagent B:** 4% (wt/v) copper (II) sulfate pentahydrate solution C2284 from Sigma-Aldrich.
- **Reagent C (freshly prepared):** Mixture of Reagent A and Reagent B in a ratio of 50 to 1 (v/v).

**Procedure:**

1. Add 60 μL α-PDL analyte solution to 1200 μL Reagent C and mix well.
2. Incubate the mixture at room temperature (≈22 °C) overnight (≈12 h).
3. Record at room temperature the absorption spectrum against Reagent C between 450 and 750 nm using a 1.5 mL, 1 cm cell (Figure 3(a)).
4. Plot the difference in absorbance in presence and absence of polylysine measured at 562 nm against the concentration of α-poly-D-Lys hydrobromide in the α-PDL analyte solution (Figure 4(b)).

**Reaction conditions (step 2 of the Procedure):**

[BCA] = 2.4 mM, [Cu(II)] = 3.0 mM, pH ≈ 11, 22 °C, 12 h reaction time.

Concentration of peptide bonds: 46 – 228 μM.

**Approximate volumes required for 50 measurements:**

- 2.0 mL 0.1 M MES buffer solution, containing 0.15 M NaCl, pH 4.7
- 1.5 mL α-PDL stock solution (2 mg/mL MES buffer solution)
- 60 mL Reagent A (BCA solution)
- 1.5 mL Reagent B (4 wt% copper(II) sulfate pentahydrate)
- 60 mL Reagent C (1:50 (v/v) mixture of Reagent A and Reagent B)