Electronic Supplementary Information (ESI) for

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

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Content:

S1: Composition BCA assay kit
S2-S3: Protocol 1: TB assay
S4-S5: Protocol 2: TNBS assay
S6-S7: Protocol 3: OPA assay
S8: Protocol 4: BCA assay

BCA assay Kit from Sigma-Aldrich (BCA1)[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 bicinchoninate, 2.0 g $Na_2CO_3 \cdot H_2O$, 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 ($CuSO_4 \cdot 5\ H_2O$) solution

4% (w/v) solution prepared in water

Product C2284 from Sigma-Aldrich

M ($CuSO_4 \cdot 5\ H_2O$) = 249.69 g/mol

CAS number of $CuSO_4 \cdot 5\ H_2O$: 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)

(2) Walker, J. M. in *The Protein Protocols Handbook*, 2nd ed., Walker, J. M., ed.; Humana Press Inc: Totowa, NJ, 2002.

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* (Figue 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 μM, pH 4.7, 37 °C, 60 min reaction time.

Concentration of lysine residues: 4.6 – 41.4 μM.

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 *α-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*)