BCA protein assay

PR-562

Test Tube Procedure

- Mix working reagent part A + part B, follow the ratio A:B = 50:1
- Pipette 50 ul working reagent into 20 ul sample and pipetting for three times
- Incubate the mixture for 10 min in room temperature
- Pipette 2ul ddH20 on the sample carrier, put the upper-portion metal down.
- Clean up sample carrier by kimwipes
- Turn on the ProNano and select BCA assay 562 nm
- Pipette 2ul working reagent for blank, and clean up by kimwipes
- Pipette 2ul reaction mixture, measure and clean up, then keep doing this step to measure all of your samples and standard protein

Drops on Parafilm Procedure -BCA protein assay (562 nm):

- Mix working reagent part A + part B, follow the ratio A:B = 50:1
- Minimum sample size is 2ul, ratio of reagent and sample is 5:2, it could be dropped on parafilm, mix and incubate on parafilm as well
- Incubate the mixture for 10 min in room temperature
- Pipette 2ul ddH20 on the sample carrier, put the upper-portion metal down.
- Clean up sample carrier by kimwipes
- Turn on the ProNano and select BCA assay 562 nm
- Pipette 2ul working reagent for blank, and clean up by kimwipes
- Pipette 2ul reaction mixture, measure and clean up, then keep doing this step to measure all of your samples and standard protein

Troubleshooting:

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt, or dilute sample. Increase Reagent B in working reagent
Blank absorbance is OK, but standards and samples show less color expected	Strong acid or alkalone buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 562 nm
All tubes are dark purple	Buffer contains a reducing agent, thiol, or biogenic amines	Dialyze or dilute sample. Remove interfering substances from sample.

Bradford assay

PR-595

Test Tube Procedure

- (a) <125 ug/ml: Pipette 10 ul working reagent into 10 ul sample and pipetting for few times

  (b) >125 ug/ml: Pipette 300 ul working reagent into 10 ul sample and pipetting for few times
- Incubate the mixture for 10 min in room temperature
- Pipette 2ul ddH20 on the sample carrier, put the upper-portion metal down.
- Clean up sample carrier by kimwipes
- Turn on the ProNano and select Bradford assay 595 nm
- Pipette 2ul reagent for blank, and clean up by kimwipes
- Pipette 2ul reaction mixture, measure and clean up
- Pipette 2ul reaction mixture, measure and clean up, then keep doing this step to measure all of your samples and standard protein

Drops on Parafilm Procedure Bradford assay (595 nm):

- (a) <125 ug/ml: Minimum sample size is 2 ul, ratio of reagent and sample is 1:1, it could be dropped on parafilm, mix and incubate on parafilm as well

  (b) >125 ug/ml: Minimum sample size is 1 ul, ratio of reagent and sample is 30:1, it could be dropped on parafilm, mix and incubate on parafilm as well
- Incubate the mixture for 10 min in room temperature
- Pipette 2ul ddH20 on the sample carrier, put the upper-portion metal down.
- Clean up sample carrier by kimwipes
- Turn on the ProNano and select Bradford assay 595 nm
- Pipette 2ul reagent for blank, and clean up by kimwipes
- Pipette 2ul reaction mixture, measure and clean up
- Pipette 2ul reaction mixture, measure and clean up, then keep doing this step to measure all of your samples and standard protein

Troubleshooting:

Problem	Possible Cause	Solution
All tubes are dark blue	Strong alkaline buffer raises pH of formulation, or sample volume too, large, thereby raising reagent pH	Dialyze or dilute sample. Remove interfering substances from sample.
Absorbance of Blank is OK, but remaining standards and samples yield lower values than expected	Improper reagent storage/	Store reagent refrigerated
	Reagent still cold	Allow reagent to warm to RT
	Absorbance measured at incorrect wavelength	Measure absorbance near 595 nm
Absorbance of Blank and standards are OK, but remaining standards and samples yield lower values than expected	Sample contains has a low molecular weight	Use the BCA or Lowry protein assay

Lowry assay

PR-660

Test Tube Procedure

- (a) <50 ug/ml: Pipette 150 ul working reagent into 20 ul sample and pipetting for few times

  (b) >50 ug/ml: Pipette 150 ul working reagent into 10 ul sample and pipetting for few times
- Incubate the mixture for 5 min in room temperature
- Pipette 2ul ddH20 on the sample carrier, put the upper-portion metal down.
- Clean up sample carrier by kimwipes
- Turn on the ProNano and select Lowry assay (660 nm)
- Pipette 2ul reagent for blank, and clean up by kimwipes
- Pipette 2ul reaction mixture, measure and clean up, then keep doing this step to measure all of your samples and standard protein

Drops on Parafilm Procedure Lowry assay (660 nm):

- (a) <50 ug/ml: Minimum sample size is 2 ul, ratio of reagent and sample is 15:2, it could be dropped on parafilm, mix and incubate on parafilm as well

  (b) >50 ug/ml: Minimum sample size is 1ul, ratio of reagent and sample is 15:1, it could be dropped on parafilm, mix and incubate on parafilm as well
- Incubate the mixture for 5 min in room temperature
- Pipette 2ul ddH20 on the sample carrier, put the upper-portion metal down.
- Clean up sample carrier by kimwipes
- Turn on the ProNano and select Lowry assay (660 nm)
- Pipette 2ul reagent for blank, and clean up by kimwipes
- Pipette 2ul reaction mixture, measure and clean up, then keep doing this step to measure all of your samples and standard protein

Troubleshooting:

Problem	Possible Cause	Solution
Standards and samples yield lower values than expected	Color measured at the wrong wavelength	Measure the absorbance at 660 nm
A precipitate forms in some tubes	Sample contains RNA/DNA	Add a final concentration of 0.8% Triton X-100 to samples
Color of samples appear darker than expected	Protein concentration is too high	Dilute sample