Protein 200 Plus Assay

Quick Reference

Assay overview

- The Protein 200 Plus LabChip® kit can be used with the Agilent 2100 bioanalyzer to analyze a large variety of protein samples, such as cell lysates, column fractions, antibodies and purified proteins.
- Ten samples can be prepared and analyzed in less than 45 minutes.
- Proteins with molecular weights from 14 – 200 kDa are separated, sized and analyzed.
- The gel-like image in figure 1 shows the name and sizes of the proteins used by the Agilent 2100 bioanalyzer to establish a standard curve to size proteins with 10 % resolution.

Sensitivity and linear range

- The assay's linear dynamic range is 20 – 2000 µg/ml, determined using BSA in PBS.
- The Protein 200 Plus LabChip kit provides staining sensitivity similar to a non-colloidal Coomassie blue stain on a 4 – 20 % gradient SDS-PAGE gel.

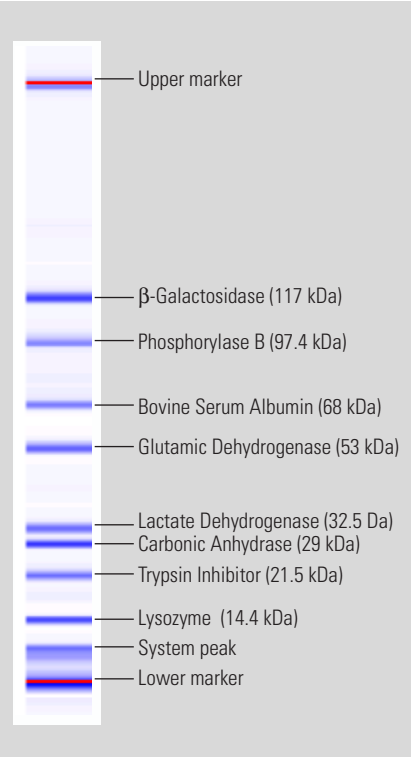

Figure 1
Gel-like image showing name and sizes of the proteins used by the Agilent 2100 bioanalyzer to establish a standard curve for protein sizing.

Possible sample types and applications

- Analysis of recombinant protein expression
- Column load/flow through comparison
- Induced/non-induced comparison
- Comparison of expression patterns
- IMAC (Immobilized Metal-Ion Affinity Chromatography) column fractions
- Reversed phase column fractions
- Purified proteins
- Antibodies
- Relative or absolute quantitation
- Assessment of relative sample purity.

Sample effects

- Sample buffers with very high salt decrease the sensitivity of the assay.
- Detergents give a large peak, which overlaps with the lower marker and might slightly affect sizing.
- Acidic buffers may affect the migration time and thus cause inaccurate sizing.

Buffer compatibility

The following table lists protein sample buffers and buffer components that are known to be compatible with the Protein 200 Plus LabChip kit and the Agilent 2100 bioanalyzer. The numbers to the right indicate effects that may occur if that particular buffer is used.

Salts and Buffers	
50 mM HEPES pH 7.5	
25 mM HEPES, 150 mM NaCl pH 7.5	
20 mM HEPES, 20 % glycerol, 0.1 M KCl, 0.2mM EDTA, 0.5 mM PMSF, 0.5 mM DTT pH 7.9	
20 mM Histidine, 30 mM NaCl, 30 mM sucrose, 290 mM glycine, 2 mM $CaCl_2$ pH 6.6	
250 mM imidazole in PBS pH 7.5	
300 mM KCl	
50 mM MES pH 6.0	
5 mM $MgCl_2$	
100 mM MOPS pH 7.2	
20 mM Na acetate	
500 mM NaCl	
25 mM NaF	
300 mM NH_4HCO_3	
200 mM NH_4SO_4	
50 mM Na or K phosphate pH 7.4	
20 mM Na phosphate, 15 mM NaCl pH 7.4	
50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole pH 7.4	
2 mM Na pyrophosphate, 1 mM Na orthovanadate, 5 mM BME, 0-500 mM imidazole pH 7.4	
26 mM NaH_2PO_4, 41 mM Na_2HPO_4, 79 mM NaCl pH 8.0	
PBS pH 7.4	
25 mM PIPES pH 7.0	
100 mM Tris-HCl pH 8.0	
100 mM Tris-bis-propane	
100 mM Tris, 3 mM desthiobiotin pH 8.0	
250 mM Tris, 20 mM glycine pH 7.5	
100 mM Tris, 150 mM Na citrate pH 7.5	
50 mM Tris, 500 mM NaCl, 0 - 500 mM imidazole pH 7.5	
18 mM Tris, 22.5 mM NaCl, 10% glycerol pH 7.5	
20 mM Tris, 500 mM NaCl, 25 mM ß-glycerophosphate pH 7.5	
50 mM Tris, 10 mM gluthathione pH 8.0	

Detergents	
1 % CHAPS in PBS pH 7.4	(1,2)
0.1 % desoxycholate in PBS pH 7.4	(3)
2 % dodecyl beta maltoside in PBS 7.4	(1)
1 % sarcosyl in PBS pH 7.4	(2,4)
1 % SDS	
1% Triton X-100	(1)
0.1 % Tween 20	(1)
0.5 % zwittergent E3-14 in PBS pH 7.4	(1,2)

Other additives	
20 % acetonitrile	(5)
10 % DMSO	
100 mM DTT	
10 mM EDTA	
20 % ethanol	
600 mM guanidine	(6)
30 % glycerol	
1 mM HCl	
5 % mannitol	
0.04 % NaN_3	
0.1 M NaOH	(7)
1 % PEG 3350 and 2000 (polyethylene glycol)	
protease inhibitor cocktail (100x diluted, Sigma)	
200 mM sucrose	
0.1 % TFA	(8)
7 M urea	

Key

1. Gives large system peak which overlaps with lower marker, slightly affects sizing.
2. Upper marker low, quantitation affected.
3. At higher concentrations: lower marker disappears, negative dip between 9-20 kD, no sizing possible.
4. Negative dip between 14-19 kDa.
5. Can precipitate SDS, must be evaporated, quantitation might be affected.
6. Precipitates SDS, quantitation might be affected.
7. Causes additional peaks in the area of the lower marker and therefore affects lower marker assignment, sizing might be affected.
8. Acidic buffers might affect sizing, must be evaporated or neutralized.

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