Agilent Protein Broad Range P240 Kit

Quick Guide

For Research Use Only. Not for use in diagnostic procedures

The Agilent ProteoAnalyzer system is an automated capillary electrophoresis platform for fast, reliable, efficient, and versatile electrophoresis of proteins.

This Quick Guide is intended for use with the Agilent ProteoAnalyzer system only. The Protein Broad Range P240 Kit is designed for the quantitative and qualitative analysis of proteins from 10 to 240 kDa.

Specifications

Analytical specifications		Protein Broad Range P240 Kit
Sizing Range	Lower Marker Only Lower Marker & Upper Marker	10 – 240 kDa 10 – 200 kDa
Typical Sizing Accuracy (% sizing error)	Lower Marker Only Lower Marker & Upper Marker	< 15% for BSA, CAII (using reduced conditions) < 10% for BSA, CAII (using reduced conditions)
Typical Resolution		<10% MWR Between 15 to 150 kDa (based on ladder) R ≥ 1 NIST mAb NGHC/HC (using reduced conditions)
Sizing Precision	Lower Marker Only Lower Marker & Upper Marker	< 8% CV for BSA, CAII, GREMLIN-1 and NIST mAb using reduced conditions < 10% CV for intact NIST mAb using non-red. conditions < 5% CV for BSA, CAII, GREMLIN-1 and NIST mAb using reduced conditions
Quantitative Range		2 ng/µL – 2000 ng/µL for BSA in PBS
Sensitivity (Signal/Noise > 3)		1 ng/µl for BSA, CAII in PBS
Quantification Reproducibility		< 25% CV for 2-20 ng/µL < 15% CV for 20-2000 ng/µL

Physical Specifications

Total electrophoresis run time	20 minutes
Samples per run	11 samples + Ladder in well 12
Sample volume required	1 µL
Kit stability	Minimum 4 months

Kit Components – 275 Sample Kit (Part# 5191-6640)

Part Number	Description	Quantity Per Kit
5191-6643	Protein Conditioning Solution, 340 mL, RT	1
5191-6644	2x Protein Inlet Buffer, 195 mL, RT Dilute with sub-micron filtered water prior to use	1
5191-6645	Protein Broad Range Gel, 120 mL, RT	1
5191-6650	Protein Fluorescent Dye, 50 µg / tube, RT *After dissolution of dye in DMF the dye must be kept at -20°C and used in ~6 weeks*	3
5191-6651	Dye Resuspension DMF, 1.5 mL, RT	1
GP-440-0100	Capillary Storage Solution, 100 mL, RT	1
5191-6647	10x Labeling Buffer, 1.1 mL, -20°C	1
5191-6648	P240 Broad Range Ladder, 30µL, -20°C	1
5191-6649	P240 Upper Marker, 30µL, -20°C	1

WARNING

- Refer to product safety data sheets for further information
- When working with the Protein Broad Range P240 Kit components follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

Additional Material Required for Analysis with the Protein Broad Range P240 Kit

- Agilent ProteoAnalyzer system with LED fluorescence detection:
- Agilent ProteoAnalyzer 12-Capillary Array, 22 cm (part# M5350-64001)

Additional equipment/reagents required (not supplied)

- 96-well Eppendorf twin tec plates. Please refer to Appendix – ProteoAnalyzer Compatible Plates in the ProteoAnalyzer System User Manual for a complete approved sample plate list
- Multichannel pipettor(s) and/or liquid handling device capable of dispensing 1 – 100 µL volumes (sample plates) and 1,000 µL volumes (inlet buffer plate)
- 0.5 mL LoBind Tubes, available from Eppendorf #022431064
- Pipette tips
- 96-well plate centrifuge (for spinning down bubbles from sample plates)
- Sub-micron filtered DI water system (for diluting the 2x Inlet Buffer)
- 1.0 M DTT (reduce freeze thaw cycles to no more than 5, store at -20°C)
- PBS: 8.1 mM Na2HPO4, 1.5 mM KH2PO4, 137 mM NaCl, 2.7 mM KCl, pH 7.4
- 1.0 M NaOH
- 0.1 M HCl
- 250 mM Iodoacetamide (46 mg/mL, prepare fresh every day)
- 96-deepwell 1 mL plate: Fisher Scientific #12-566-120 (inlet buffer and/or waste plate)
- Reagent reservoir, 50 mL (VWR #89094-680 or similar) (for use in pipetting inlet buffer plates/sample trays)
- Conical centrifuge tubes for Protein Broad Range Gel and/or Protein Conditioning Solution
- 50 mL BD Falcon #352070, available from Fisher Scientific #14-432-22 or VWR #21008-940
- Vortexer (for mixing of samples, ladder, and/or labeling buffer in tubes and/or plates)
- Heat block or Thermocycler

Essential Measurement Practices

Environmental conditions	• Ambient operating temperature: 18 – 25 °C (64 – 77 °F) • Keep reagents at room temperature during sample preparation
Steps before sample preparation	• Allow reagents to equilibrate at room temperature for 30 min prior to use • Room Temp reagents should be allowed to equilibrate to room temp for 24 hrs. upon initial receipt of reagents
Pipetting practice	• Pipette reagents carefully against the side of the 96-well sample plate or sample tube • Ensure that no sample/ladder or Master Mix remains within or on the outside of the tip
Mixing and centrifugation recommendations	• Apply a new seal to 96-well sample plate prior to mixing and centrifugation • Gently invert reagent bottles 4-5x prior to aliquoting • When mixing sample with Master Mix (MM), it is important to mix the contents of the well thoroughly to achieve the most accurate quantification. After adding 1 µL of sample or ladder to the 29 µL of MM, place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm for 2 min. Any suitable benchtop plate vortexer can be used. Ensure that there is no well-to-well transfer of samples when vortexing. The plate should be spun via a centrifuge after vortexing to ensure there are no trapped air bubbles in the wells • After mixing and centrifuging visually confirm that all liquid is collected at the bottom of the 96-well sample plate and any air bubbles are removed • Once the plate is prepared place on the instrument for analysis and remove plate seal
Optimization of sample preparation	• In some cases, the temperature and time of the heating step for protein denaturation may need to be adapted depending on the sample type. E.g. For many antibodies it is recommended to reduce the incubation temperature from 85°C to 70°C to avoid method-induced degradation. For stable proteins it might be necessary to increase incubation temperature to 95-100°C to achieve complete denaturation
Optimization of injection conditions	• The default injection time and injection voltage can be altered when needed. In cases where peaks are too high and a Warning 'Peak height above RFU threshold' is seen, injection time or voltage may need to be lowered. When peak heights are too low then injection time or voltage may need to be increased. It is recommended to change the injection time before changing the voltage. We do not recommend raising the injection voltage above 9 kV

Solutions preparation

ProteoAnalyzer System Volume Specifications

# of Samples	Protein Broad Range Gel Volume (mL)
12	10
24	15
48	25
96	45

# of Samples	Volume of Protein Conditioning Solution (mL)
12	10
24	15
48	25
96	45

[1] One sample well per separation is dedicated to the ladder.
[2] A 5 mL minimum dead volume in the conical is included. ~4 mL of gel is consumed per run.
[3] **Do not** discard unused gel after analysis. The gel remaining in the conical must be used to achieve analysis of 275 samples.

Capillary Array Cleaning

1. Once a day, prior to running samples, it is important to run the Daily Conditioning Flush Flush (10 min 1 M NaOH; 3 min Protein Conditioning solution)

Reagent Preparation

1. Pour fresh Protein Broad Range gel according to daily need. Refer to volume specifications table for volume needed based on number of samples.
2. Refill Protein Conditioning Solution as needed. Refer to volume specifications table for volume needed based on number of samples.
3. Fill row A of a deep well plate with 1x Protein Inlet Buffer in drawer 'B' on the system, 1.0 mL/well. Replace daily.
4. Fill row B of the deep well plate with 1mL/well of DI water in drawer "B" on the system. Replace daily.
5. Fill row H of the deep well plate with Capillary Storage Solution, 1.0mL/well.
   Place in drawer "B."
   - Replace every 2 weeks. Do not top off.

Labeling Workflow

1. Add 44 µL Dye Resuspension DMF to one Protein Fluorescent Dye tube, vortex 30 s and centrifuge briefly. Once resuspended the dye must be stored at -20 °C and used within 6 weeks.
2. Dilute ladder (1 µL stock + 3 µL sample matrix, to improve sizing accuracy), vortex and centrifuge briefly.
3. If using the P240 Upper Marker; dilute 1 µL stock + 5.7 µL DI water, mix and centrifuge briefly.
4. Prepare a labeling Master Mix in a 0.5 mL Protein LoBind vial according to the following protocol.

   These volumes are for 12 samples (1 row). For additional rows, multiply each volume by the number of rows.

   For Reducing Conditions
   - 381 µL DI Water
   - 45 µL 10x Labeling Buffer
   - 4.5 µL 1.0 M DTT
   - 3.9 µL Resuspended Protein Fluorescent Dye
   - 0.9 µL Diluted P240 Upper Marker (If needed)

   For Non-Reducing Conditions
   - 340 µL DI Water
   - 45 µL 10x Labeling Buffer
   - 45 µL 250 mM Iodoacetamide
   - 3.9 µL Resuspended Protein Fluorescent Dye
   - 0.9 µL Diluted P240 Upper Marker (If needed)

   *Important: Thoroughly vortex and centrifuge Master Mix. Use the Master Mix in the labeling reaction in a timely manner.

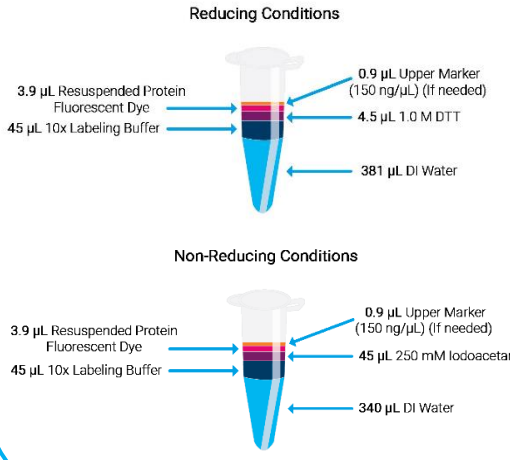
5. Pipette 29 µL of labeling Master Mix into wells 1-12 of sample plate. Add 1 µL of sample to wells 1-11 and 1 µL of diluted P240 Broad Range Ladder to well 12.
6. Seal plate and vortex for 2 min at 3000 rpm.
7. Centrifuge plate for 20 seconds.
8. Place on heating block/thermocycler and incubate for 10 min at 85°C. (Should be a noticeable change in color after heating). Optimum conditions may vary by sample type. See Optimization of Sample Preparation section.
9. Take plate off heating block/thermocycler and allow to cool for 5 min at RT.
10. DO NOT vortex plate after it has cooled as this will introduce unwanted bubbles.
11. Centrifuge plate for 20 seconds, remove seal, and place onto instrument.

1 Add 44 µL Dye Resuspension DMF to one Protein Fluorescent Dye tube, vortex 30 s and centrifuge briefly. Once resuspended the dye must be stored at -20 °C and used within 6 weeks.

2 Dilute ladder (1 µL stock + 3 µL sample matrix, to improve sizing accuracy), vortex and centrifuge briefly.

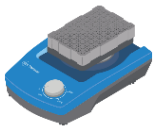
3 If using the P240 Upper Marker; dilute 1 µL stock + 5.7 µL DI water, mix and centrifuge briefly.

4 Prepare a labeling Master Mix in a 0.5 mL Protein LoBind vial according to the following protocol.



5 Pipette 29 µL of labeling Master Mix into wells 1-12 of sample plate. Add 1 µL of sample to wells 1-11 and 1 µL of diluted P240 Broad Range Ladder to well 12.

6/7 Seal plate and vortex for 2 min at 3000 rpm then centrifuge plate for 20 seconds.


Seal plate & Vortex


Spin Down

8/9/10/11 Place on heating block/thermocycler and incubate for 10 min at 85°C. (Color should change from blue to yellowish/reddish color after heating). Optimum conditions may vary by sample type. Take plate off heating block/thermocycler and allow to cool for 5 min at RT.

DO NOT vortex plate after it has cooled as this will introduce unwanted bubbles. Centrifuge plate for 20 seconds,

WARNING

Working with Chemicals
The handling of reagents and chemicals might hold health risks.

- Refer to product material safety datasheets for further chemical and biological safety information.
- Follow the appropriate safety procedures such wearing Personal Protective Equipment (PPE).

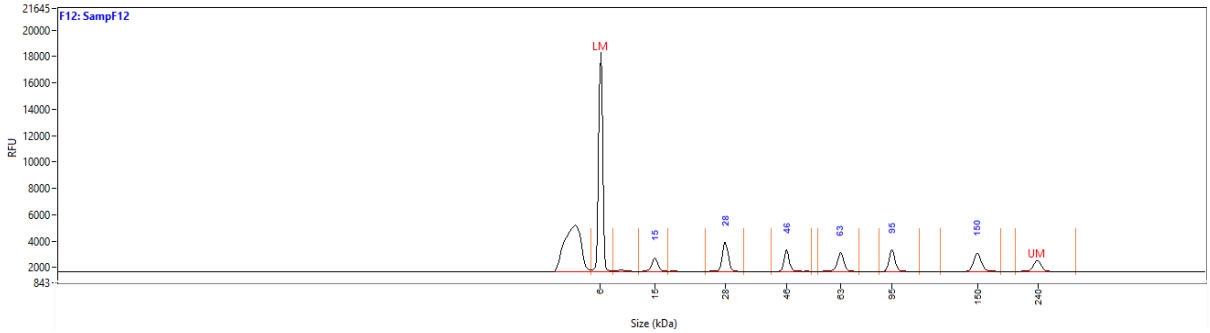
Agilent ProteoAnalyzer software operating procedure

1. Select row to run.
2. Enter **sample ID** and **Tray ID** (optional).
3. Select **Add to Queue**, from the dropdown menus select the corresponding method.
   - 5191-6640UM_LM – ProteoAnalyzer Broad Range Kit LM and UM

- 5191-6640LM – ProteoAnalyzer Broad Range Kit LM only

4. Enter **Tray Name**, **Folder Prefix**, and **Notes** (optional).
5. Select **OK** to add method to the queue.
6. Select  to start the separation.

P240 Broad Range Protein Ladder result



Representative result of the P240 Broad Range Protein Ladder diluted 1:4 in PBS with reducing conditions on the ProteoAnalyzer system with the Agilent Protein Broad Range P240 Kit using the Lower and Upper Markers.

Troubleshooting

The following table lists potential assay specific issues which may be encountered when using the Agilent P240 Broad Range Protein Kit (part# 5191-6640) and suggested remedies. Contact electrophoresis@agilent.com if you have any additional troubleshooting or maintenance questions.

Issue	Cause	Corrective Action
Broad Peaks and/or slow migration	1. Improperly conditioned capillary	1. Method C 1.0 M NaOH Flush and if problem persists Method B 0.1 M HCl Flush followed by daily conditioning flush
Intermittent Current Instability, No peaks or low signal	1. Clogged Capillary	1. Method A: Hot Water Soak followed by Method C 1.0 M NaOH Flush
Repeated Current Instability, No peaks or low signal	1. Air Bubble 2. Partially Clogged Capillary	1. Centrifuge Sample Plate and Repeat Run 2. Method A: Hot Water Soak followed by Method C 1.0 M NaOH Flush
Crosstalk Between Capillaries	1. Peaks >25,000 RFU may cause crosstalk between capillaries	1. Skip a capillary when running samples that produce a signal >25,000 RFU
Warning 'Peak height above RFU threshold'	1. Signal larger than ≈ 63,000 RFU	1. Lower sample concentration or decrease injection time or voltage. Refer to Optimization of injection conditions section above
Dynamic range of 3 logs not obtained, low sensitivity	1. Signal too low 2. Sample buffer compatibility issue (e.g. high salt concentration)	1. Increase sample concentration or injection time or voltage. Refer to Optimization of injection conditions section above 2. Refer to Sample Buffer Compatibility List below

Additional peaks, sample purity and/or resolution lower than expected	1. Method-induced degradation 2. Incomplete denaturation	1. Reduce temperature and/or time of the heating step during sample labeling. See Optimization of Sample Preparation section. 2. Increase temperature and/or time of the heating step during sample labeling. See Optimization of Sample Preparation section.
Quantification results outside of Specification	1. Incorrect assignment of LM 2. Sample buffer compatibility issue	1. Check assignment of LM, assign LM manually* 2. Refer to Sample Buffer Compatibility List below
Sizing results outside expectations	1. Incorrect assignment of LM and/or UM 2. Incorrect assignment of Ladder peaks 3. Sample buffer compatibility issue 4. Protein characteristics (pI, hydrophobicity, PTMs)	1. Check assignment of markers, assign markers manually*, include an UM 2. Check assignment of Ladder, assign peaks manually* 3. Dilute Ladder in sample buffer, refer to Sample Buffer Compatibility List below

*see also ProSize Data Analysis Software User Manual page 40

Sample Buffer Compatibility List

The following table lists protein sample buffers and buffer components which are known to have a low impact on the labeling reaction. Others have a strong negative influence.

Low Impact: 50% - 300% signal compared to PBS *	Comment
200 mM Tris-HCl, pH 8.0	
50 mM Citrate buffer pH 4	
50 mM Acetate buffer pH 5.2	
50 mM MES pH 6	
50 mM MOPS pH 7	
50 mM HEPES, pH 8.0	
100 mM $NaHCO_3$ pH 8.5	
7 M urea or 2 M thiourea	
5% 2-Mercaptoethanol	
20 mM DL-Dithiothreitol (Cleland's Reagent, DTT)	
50 mM $MgCl_2$	
100 mM KCl	Higher concentrations of potassium are not recommended due to the low solubility of potassium dodecyl sulfate
1% Triton X-100	
1% Tween 20	
4% CHAPS	High CHAPS concentrations effect sizing, the use of Ladder diluted in sample buffer is recommended
1% NP-40	
1% SDS	
300 mM NaCl	If the salt concentration in the sample is higher than 300 mM a buffer exchange to a lower ionic strength buffer is recommended
Strong Impact: < 50% signal compared to PBS *	**Comment**
50 mM Tris(2-carboxyethyl)phosphine (TCEP)	Incompatible with fluorescent dye

* Phosphate Buffered Saline: 8.1 mM Na2HPO4, 1.5 mM KH2PO4, 137 mM NaCl, 2.7 mM KCl, pH 7.4

Reducing agents

Reagent	Comment
10 mM DL-Dithiothreitol (Cleland's Reagent, DTT)	Recommended
5% 2-Mercaptoethanol	3-5 fold signal decrease in comparison to 10 mM DTT
50 mM Tris(2-carboxyethyl)phosphine (TCEP)	Not recommended due to incompatibility with fluorescent dye

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Technical Support and Further Information

For technical support, please visit www.agilent.com. It offers useful information and support about the products and technology

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