# Agilent dsDNA 910 Reagent Kit (35-1500 bp)

# Quick Guide

***For Research Use Only. Not for use in diagnostic procedures***

This Quick Guide is intended for use with the Agilent 5200, 5300, and 5400 Fragment Analyzer systems only. The dsDNA 910 Reagent kit from Agilent is for the analysis of dsDNA fragments between 35 bp and 1,500 bp. Sizing and relative quantification of samples can be obtained using this kit. Example applications include general PCR fragment sizing and QC, and genotyping.

## Specifications

| Analytical Specifications | dsDNA 910 Reagent Kit |
|---|---|
| DNA Sizing Range | 35 – 1,500 bp |
| DNA Sizing Accuracy[1] | ± 5% or better |
| DNA Sizing Precision[1] | 2% CV |
| Separation Resolution | 35 bp – 100 bp ≤ 10%: 100 bp – 700 bp ≤ 5%; 700 bp – 1,500 bp ≤10% (ultrashort capillary array, 22 cm)<br>35 bp – 100 bp ≤ 10%; 100 bp – 1,000 bp ≤ 5%; 1,000 bp – 1,500 bp ≤ 10% (short capillary array, 33 cm)<br>35 bp – 100 bp ≤ 10%; 100 bp – 1,500 bp ≤ 5% (long capillary array, 55 cm) |
| Fragment Concentration Range[1] | 0.5 ng/µL – 50 ng/µL input DNA (adjustable by dilution sample) |
| **Physical Specifications** | |
| Total Electrophoresis Run Time | 22cm[2]: 22 minutes, 33cm: 45 minutes, 55cm: 70 minutes |
| Samples Per Run | 12, 48 or 96; depending on the instrument type |
| Sample Volume Required | 2 µL (adjustable depending on sample concentration) |
| Guaranteed Shelf Life | 4 months |

1 Results using DNA Ladder or DNA Fragment standards initially prepared in 1X TE buffer.
2 The FA 12-Capillary Array Ultrashort, 22 cm is only available for the 5200 Fragment Analyzer system.

Kit Components – 500 Sample Kit – *Refer to product label for proper storage conditions*

| Kit Component Number | Part Number (Re-order Number) | Description | Quantity Per Kit |
|---|---|---|---|
| 5191-6595* | | dsDNA 910/915, 500, 4°C | |
| | DNF-810-0240 | dsDNA 810 Gel, 240 mL | 1 |
| | DNF-355-0125 | 5x 930 dsDNA Inlet Buffer, 125 mL | 1 |
| | DNF-495-0060 | Dilution Buffer 1X TE, 60 mL | 1 |
| DNF-910-FR* | | dsDNA 910 (35-1500 bp) FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 1 |
| | FS-SLR910-0001 | 100 bp DNA Ladder, 1 mL | 1 |
| | FS-SMK910-0003 | Markers, 35 bp & 1500 bp, 3.2 mL | 1 |
| 5191-6614* | | Qualitative DNA, 500, RT | |
| | FS-SMO15 | Mineral Oil Dropper Bottle, 15 mL | 1 |
| | DNF-475-0050 | 5x Capillary Conditioning Solution, 50 mL | 1 |

Kit Components – 1000 Sample Kit – *Refer to product label for proper storage conditions*

| Kit Component Number | Part Number (Re-order Number) | Description | Quantity Per Kit |
|---|---|---|---|
| 5191-6596* | | dsDNA 910/915, 1000, 4°C | |
| | DNF-810-0500 | dsDNA 910 Gel, 500 mL | 1 |
| | DNF-355-0300 | 5x 930 dsDNA Inlet Buffer, 300 mL | 1 |
| | DNF-495-0125 | Dilution Buffer 1X TE, 125 mL | 1 |
| DNF-910-FR* | | dsDNA 910 (35-1500 bp) FR | |
| | DNF-600-U030 | Intercalating Dye, 30 µL | 2 |
| | FS-SLR910-0001 | 100 bp DNA Ladder, 1 mL | 2 |
| | FS-SMK910-0003 | Markers, 35 bp & 1500 bp, 3.2 mL | 2 |
| 5191-6615* | | Qualitative DNA, 1000/5000, RT | |
| | FS-SMO15 | Mineral Oil Dropper Bottle, 15 mL | 1 |
| | DNF-475-0100 | 5x Capillary Conditioning Solution, 100 mL | 1 |

*not orderable

Altering any reagents and/or use of unapproved or non-recommended reagents may materially alter the performance of the instrument such that the instrument no longer performs to Agilent specifications. Any work performed by Agilent to bring the instrument back into compliance with Agilent specifications will be performed at the customer's expense.

## Additional Material Required for Analysis with Fragment Analyzer Systems (not supplied)

| Instrument | Compatible Arrays | Part Number |
|---|---|---|
| 5200 Fragment Analyzer | FA 12 Capillary Array Ultrashort<br>FA 12 Capillary Array Short<br>FA 12 Capillary Array Long | A2300-1250-2247<br>A2300-1250-3355<br>A2300-1250-5580 |
| 5300 Fragment Analyzer. | FA 48 Capillary Array Short<br>FA/ZAG 96 Capillary Array Short<br>FA/ZAG 96 Capillary Array Long | A2300-4850-3355<br>A2300-9650-3355<br>A2300-9650-5580 |
| 5400 Fragment Analyzer | FA/ZAG 96 Capillary Array Short<br>FA/ZAG 96 Capillary Array Long | A2300-9650-3355<br>A2300-9650-5580 |

### Software

- Fragment Analyzer controller software
- ProSize data analysis software

### Reagents

- Capillary Storage Solution (GP-440-0100)

## Additional equipment required (not supplied)

- 96-well PCR sample plates (*Refer to Appendix in Fragment Analyzer User Manual*)
- Multichannel pipettor and/or liquid handling device capable of dispensing 1-100 µL (sample plates) and 1,000 µL (inlet buffer plate)
- Pipette tips
- 96-well plate centrifuge
- Adhesive PCR plate seals
- Sub-micron filtered DI water system: for dilutions
- 96-deepwell 1 mL plate: inlet buffer and/or waste plate (Agilent #P60-20 or Fisher Scientific #12-566-120)
- Reagent reservoir 50 mL: for use in pipetting inlet buffer plates (VWR #89094-680, or similar)
- Conical centrifuge tubes for prepared separation gel+dye mixture and/or 1x Capillary Conditioning Solution
  - 50 mL for 5200 Fragment Analyzer system (BD Falcon #352070, Fisher Scientific #14-432-22 or VWR #21008-940)
  - 250 mL for 5300 and 5400 Fragment Analyzer systems (Corning #430776, Fisher Scientific #05-538-53 or VWR #21008-771)
- Vortexer

WARNING

**Working with Chemicals**

- **Refer to product safety data sheets for further information**
- **When working with the Fragment Analyzer kit components follow the appropriate safety procedures such as wearing personal protective equipment (PPE).**

## Essential Measurement Practices

| | |
|---|---|
| **Environmental conditions** | • Ambient operating temperature: 19 – 25 °C (66 – 77 °F)<br>• Keep insturment reagents at room temperature during sample preparation. |
| **Sample Input Concentration** | • Ensure sample input concentrations lie within kit specifications.<br>• Sample signal should not exceed 60,000 RFU. |
| **Steps before sample preparation** | • Allow instrument reagents to equilibrate at room temperature for 30 min prior to use. |
| **Pipetting practice** | • Pipette reagents against the side of the 96-well sample plate or sample tube.<br>• Ensure no sample, dilution buffer or Marker remains within or on the outside of the tip. |
| **Marker Plate Preparation** | • Bring ready-to-use Marker solution to room temperature prior to use.<br>• Agitate and centrifuge ready-to-use Marker solution to mix.<br>• Dispense 30 µL/well of Marker solution into wells of a separate sample plate. (12 capillary; row A, 48 capillary; rows A-D, 96 capillary; all rows)<br>• Cover the wells with 1 drop or 30 µL of mineral oil to allow reuse for 30+ injections or ~ 1 month.<br>• Place plate in drawer M. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate. |
| **Sample Plate Preparation** | • Pipette 22 µL of 1x TE dilution buffer to each well in a row that will contain sample.<br>• If running the DNA Ladder with samples, pipette 24 µL Ladder solution (ready-to-use) directly into the specified well of the sample plate or row to be analyzed.<br>• Pipette 2 µL of each sample into the 22 µL of 1x TE dilution buffer in the respective wells of the sample plate. Mix contents of the well using a pipette by aspiration or expulsion in the pipette tip.<br>• Place a plate seal on the sample plate and vortex the sample plate at 3,000 rpm or 2 minutes. Ensure there is no well-to-well transfer of samples when vortexing.<br>• After mixing, centrifuge the plate to remove any air bubbles.<br>• Run samples immediately after preparation, or within a day with oil overlay. If not using right away, cover and keep at 4°C, warm to RT and centrifuge before running plate. |

### Gel Preparation

Centrifuge dye prior to opening the vial to reduce risk of leaking. Ensure the gel + dye is mixed without generating bubbles, gently invert tube 5-10 times.

| Number of Samples | Intercalating Dye Volume (µL) | Separation Gel Volume (mL) |
|---|---|---|
| 12 | 1 | 10 |
| 24 | 1.5 | 15 |
| 48 | 2.5 | 25 |
| 96 | 4.5 | 45 |
| 192 | 8 | 80 |
| 384 | 16 | 160 |

### Conditioning Solution

The provided 5X Conditioning Solution must be diluted to 1X using submicron DI water prior to use. Invert to mix.

| Number of Samples | Volume of 1X Conditioning Solution (mL) |
|---|---|
| 12 | 10 |
| 24 | 15 |
| 48 | 25 |
| 96 | 45 |
| 192 | 80 |
| 384 | 160 |

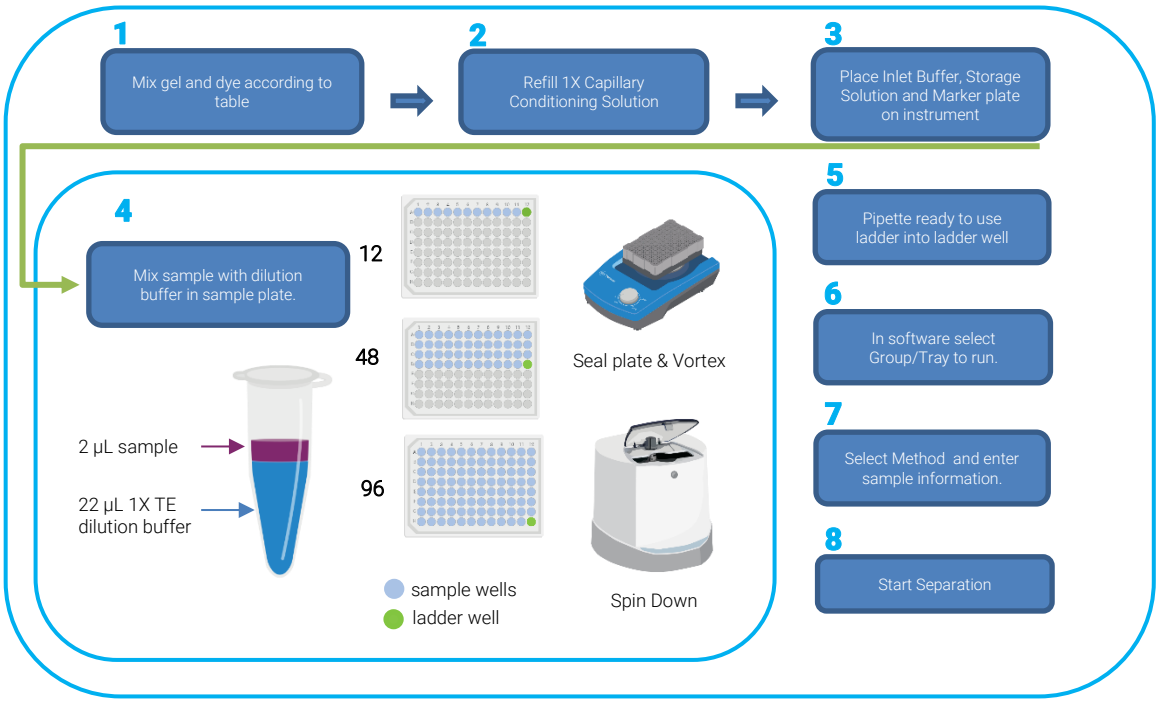
## Agilent dsDNA 910 Reagent Kit Operating Procedure

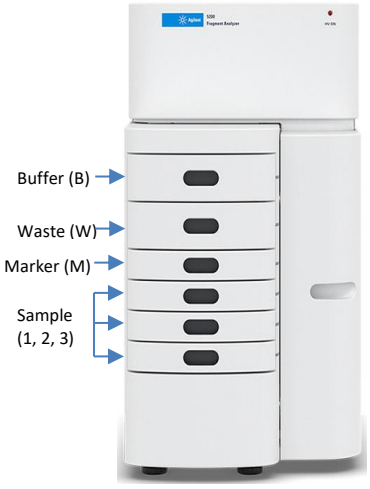
1. Mix fresh gel and dye according to the volumes in the preparation table. Update solution level in controller software.
2. Refill 1X Capillary Conditioning Solution as needed. Update solution level in controller software.
3. Inspect and empty, if necessary, waste plate located in drawer "W".
4. Place a fresh 1X Inlet Buffer tray, 1 mL/well, in drawer "B". Replace daily.
   - 5200 – row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows

   Prepare Capillary Storage Solution plate. Replace every 2 weeks for optimal results.
   - 5200 – row H, 1 mL/well, drawer B
   - 5300 – 48 capillary, rows A-D, 100 µL/well, drawer 3
   - 5300/5400 – 96 capillary, all rows, 100 µL/well, drawer 3

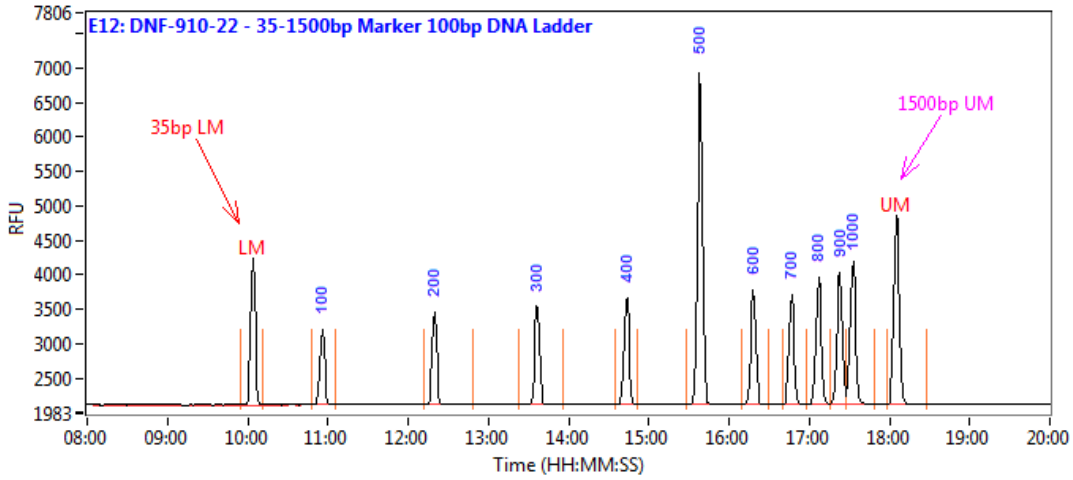
   Prepare Marker plate and place in drawer "M", 30 µL/ well. Add 1 drop or ~30 µL of mineral oil to each well. The marker plate should last for 30+ injections or ~1 month.
   - 5200 – Row A
   - 5300 – 48 capillary, rows A-D
   - 5300/5400 – 96 capillary, all rows
5. Mix samples with 1x TE diluent buffer in sample plate. Refer to sample plate preparation.
6. Add ready to use ladder in corresponding well (see sample plate image below), depending on capillary array used.
7. Select Row/Group/Tray to run. Enter sample ID and Tray ID, if desired.
8. Add to queue, from the dropdown select the corresponding method based on your capillary length;
   - DNF-910-22
   - DNF-910-33
   - DNF-910-55

   Enter Tray Name, Folder Prefix and Notes, if desired.
9. Add method to the queue by selecting "OK", press play to start the separation.



100 bp DNA Ladder result




Representative 100 bp DNA Ladder result double injected with 35 bp lower marker and 1,500 bp upper marker, using the Fragment Analyzer system with the dsDNA 910 Reagent Kit. Method: DNF-910-22 ("ultra-short" array). RFU values may differ between instruments.

## Troubleshooting

The following table lists several potential kit specific issues which may be encountered when using the dsDNA 910 Reagent Kit and suggested remedies. Contact Agilent technical support if you have any additional troubleshooting or maintenance questions.

| Issue | Cause | Corrective Action |
|---|---|---|
| The peak signal is >> 60,000 RFU; upper marker peak is low or not detected relative to lower marker. | 1 Input DNA sample concentration is too high. | 1 Further dilute input DNA sample concentration with 1x TE buffer and repeat experiment. |
| Sample peak(s) migrate before or co-migrate with 35 bp Lower Marker. | 1 Excess primer-dimer species in sample. | 1 Further dilute input DNA sample concentration with 1x TE buffer to minimize primer-dimer interference and repeat experiment. |
| Sample peak(s) migrate after or co-migrate with 1,500 bp Upper Marker. | 1 DNA sample size out of range of kit. | 1 Analyze samples with dsDNA 915 Reagent Kit, 35 bp – 5,000 bp, dsDNA 920 Reagent Kit, 75 bp – 15,000 bp, or dsDNA 930 Reagent Kit, 75 bp –20,000 bp. |
| No peak observed for DNA sample when expected. Lower/Upper Marker peaks observed. | 1 Sample concentration too low and out of range. | 1 Prepare more concentrated sample and repeat experiment (e.g., 4 µL sample + 20 µL 1x TE buffer). |
| | 2 Sample was not added to 1x TE dilution buffer or not mixed well. | 2 Verify sample was correctly added and mixed to sample well. |
| No sample peak or marker peak observed for individual sample. | 1 Air trapped at the bottom of sample plate well, or bubbles present in sample well. | 1 Check sample plate wells for trapped air bubbles. Centrifuge plate. |
| | 2 Insufficient sample volume. A minimum of 20 µL is required. | 2 Verify proper volume of solution was added to sample well. |
| | 3 Capillary is plugged. | 3 Check waste plate for liquid in the capillary well using a 96-deepwell plate. If no liquid is observed, follow the steps outlined in the System Manual for unclogging a capillary array. |

**For Research Use Only**
Not for use in diagnostic procedures.

**Technical Support and Further Information**
For technical support please visit www.agilent.com which offers useful information and support regarding the products and technology.

www.agilent.com



SD-AT000121 Edition 03/25