

## **GE Superdex 200 10/300 GL**

### **Gel Filtration Calibration protocol**

#### **I. Protein Mixture (Standards)**

| <b><u>No.</u></b> | <b><u>PROTEIN NAME</u></b> | <b><u>SIZE</u></b> | <b><u>Concentration</u></b> |
|-------------------|----------------------------|--------------------|-----------------------------|
| 1                 | Blue Dextran               | 2000 KDa           | N/A                         |
| 2                 | Thyroglobulin              | 669 KDa            | 35 mg/mL                    |
| 3                 | Ferritin                   | 450 KDa            | 17                          |
| 4                 | Catalase                   | 67 KDa             | 10                          |
| 5                 | Ovalbumin                  | 43 KDa             | 10                          |
| 6                 | Carbonic Anhydrase         | 29 KDa             | 10                          |
| 7                 | Cytochrome C               | 13.6 KDa           | 10                          |
| 8                 | Aprotinin                  | 6.5 KDa            | 10                          |
| 9                 | Tryptophan                 | 250 Da             | 30mM                        |

1. Prepare protein standards in 50mM Sodium phosphate, 150mM NaCl, pH-7.2
2. Blue Dextran is used to calculate the column void volume ( $V_0$ ). It should always be prepared fresh with 1mg/mL concentration. Rate of solubilization of Blue dextran 2000 may be increased by heating the buffer to 50°C before adding blue dextran.
3. Proteins are stored in Proteins Reagent Box at 4°C.
4. Use 1:1 Volume ratio of all the eight proteins (#2-9) to make protein mix.

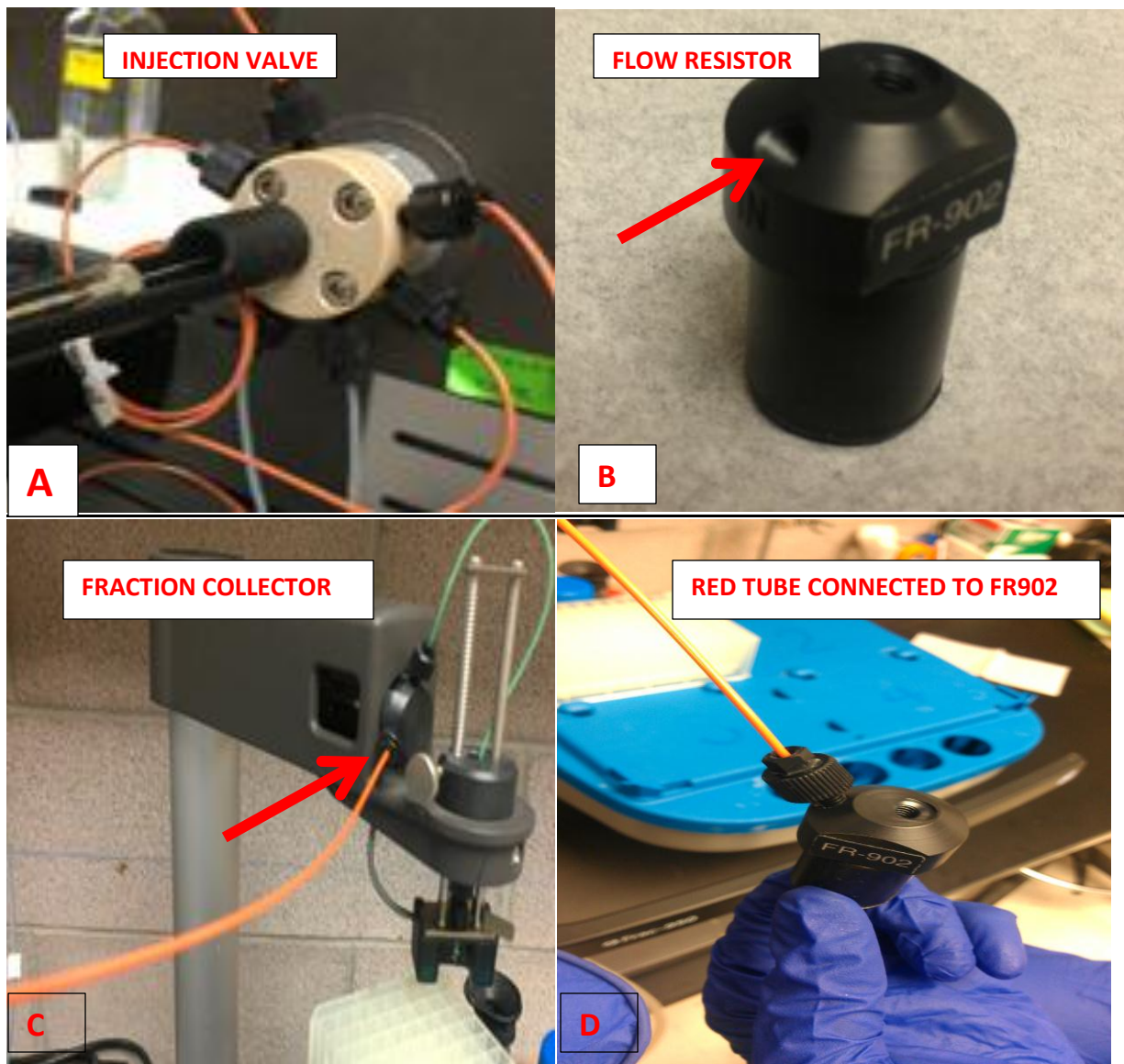
Refer: GE Filtration Calibration Kit Manual.

#### **II. Connecting Superdex Column**

Whenever you unplug any tubing at the injection valve (FigA), Use flow restrictor (FR-902, in FPLC drawer, Fig B) to ensure no bubbles are being introduced in the system.

1. Connect the orange tube (going in the fraction collector, shown in Fig. C) at the "IN" of FR-902, shown in Fig.D
2. Then, connect each tube one at a time.
3. If FR-902 is not used while changing the tubes at Injection valve, bypass the column, and run 5-10mL/min flowrate, for 5-10min (creates pressure to remove bubbles from the tubes).

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### **III. Running the Supderdex Protocol**

1. Use 50uL of each protein from (#2-9). Dilute it with 400uL of 1X PBS buffer.
2. Inject 500uL of the diluted protein standard.
3. Empty loop with 1ML of 1X PBS
4. Use Method: Superloop20020mL to collect fractions in 96-well plate.

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5. To check the elution profile of the proteins Refer to Result file “110208Superdex200MRSstandards001” in UNICORN software.

**Sample recommendations**

|                                  |                                                                                                            |
|----------------------------------|------------------------------------------------------------------------------------------------------------|
| Molecular weight, M <sub>r</sub> | 3 000–70 000 (Superdex 75)<br>10 000–600 000 (Superdex 200)                                                |
| Protein concentration            | ≤ 10 mg in sample                                                                                          |
| Sample volume                    | 25–500 µl                                                                                                  |
| Preparation                      | Dissolve the sample in eluent,<br>filter through a 0.22 µm filter or<br>centrifuge at 10 000 g for 10 min. |



**Table 1.** The main characteristics of Superdex media

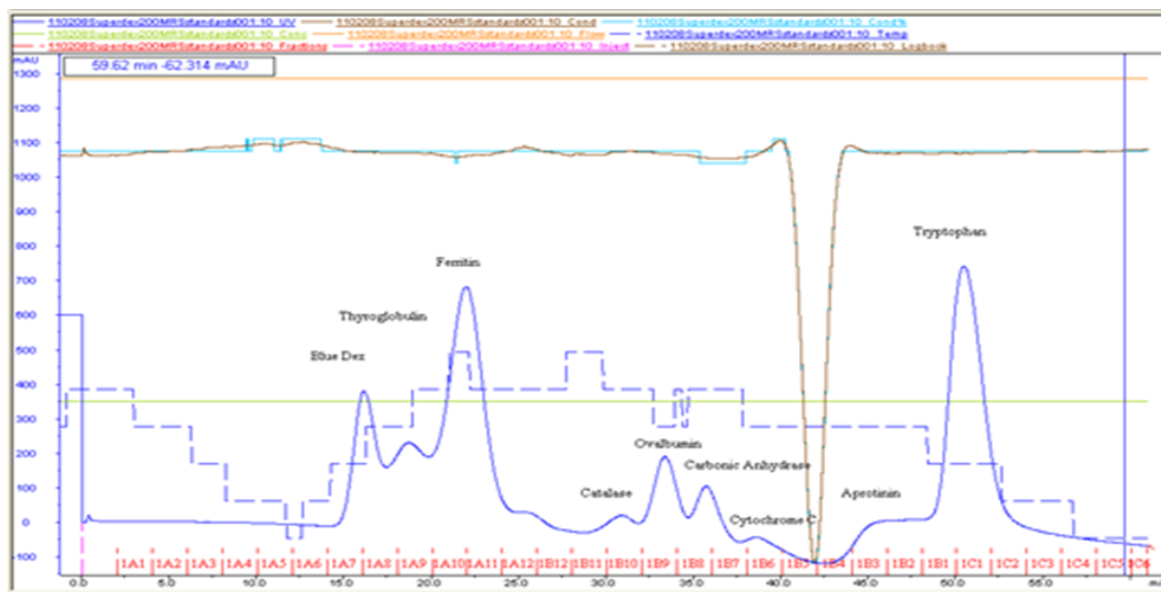
|                                    | Superdex Peptide                                        | Superdex 75                                             | Superdex 200                                            |
|------------------------------------|---------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------|
| Separation range (M <sub>r</sub> ) | 100 to 7 000 (peptides)                                 | 3 000 to 70 000 (globular proteins)                     | 10 000 to 600 000 (globular proteins)                   |
| Exclusion limit (M <sub>r</sub> )  | 20 000                                                  | 100 000 (globular proteins)                             | 1 300 000 (globular proteins)                           |
| pH stability                       | 1 to 14 (long- and short-term)                          | 3 to 12 (long-term),<br>1 to 14 (short-term)            | 3 to 12 (long-term),<br>1 to 14 (short-term)            |
| Temperature stability              | 4°C to 40°C                                             | 4°C to 40°C                                             | 4°C to 40°C                                             |
| Working and storage temperature    | 4°C to 30°C                                             | 4°C to 30°C                                             | 4°C to 30°C                                             |
| Matrix                             | Spherical composite of cross-linked agarose and dextran | Spherical composite of cross-linked agarose and dextran | Spherical composite of cross-linked agarose and dextran |
| Average particle size              | 13 µm                                                   | 13 µm                                                   | 13 µm                                                   |

([https://www.gelifesciences.com/gehcls\\_images/GELS/Related%20Content/Files/1314807262343/litdoc71501796AG\\_20110831221234.pdf](https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314807262343/litdoc71501796AG_20110831221234.pdf))

#### **IV. Elution Profile of GF Calibration Protein Standards**

| <b>No.</b> | <b>PROTEIN NAME</b> | <b>SIZE</b> | <b>Elution Time (min)</b> |
|------------|---------------------|-------------|---------------------------|
| 1          | Blue Dextran        | 2000 KDa    | 16.1                      |
| 2          | Thyroglobulin       | 669 KDa     | 18.6                      |
| 3          | Ferritin            | 450 KDa     | 22                        |
| 4          | Catalase            | 67 KDa      | 30.49                     |
| 5          | Ovalbumin           | 43 KDa      | 33.06                     |
| 6          | Carbonic Anhydrase  | 29 KDa      | 35.99                     |
| 7          | Cytochrome C        | 13.6 KDa    | 38.35                     |
| 8          | Aprotinin           | 6.5 KDa     | 45.5                      |
| 9          | Tryptophan          | 250 Da      | 50.4                      |

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| <u>Superdex 200 Time Elution</u> | <u>Log MW</u> | <u>MW</u> |
|----------------------------------|---------------|-----------|
| 18                               | 6.0           | 940806    |
| 19                               | 5.9           | 775532    |
| 20                               | 5.8           | 639293    |
| 21                               | 5.7           | 526987    |
| 22                               | 5.6           | 434410    |
| 23                               | 5.6           | 358096    |
| 24                               | 5.5           | 295189    |
| 25                               | 5.4           | 243332    |
| 26                               | 5.3           | 200586    |
| 27                               | 5.2           | 165348    |
| 28                               | 5.1           | 136301    |
| 29                               | 5.1           | 112357    |
| 30                               | 5.0           | 92619     |
| 31                               | 4.9           | 76348     |
| 32                               | 4.8           | 62936     |
| 33                               | 4.7           | 51880     |
| 34                               | 4.6           | 42766     |
| 35                               | 4.5           | 35253     |
| 36                               | 4.5           | 29060     |
| 37                               | 4.4           | 23955     |
| 38                               | 4.3           | 19747     |
| 39                               | 4.2           | 16278     |
| 40                               | 4.1           | 13418     |
| 41                               | 4.0           | 11061     |
| 42                               | 4.0           | 9118      |
| 43                               | 3.9           | 7516      |
| 44                               | 3.8           | 6196      |
| 45                               | 3.7           | 5107      |
| 46                               | 3.6           | 4210      |

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