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## Specification Sheet

### nanoHA<sub>10-20</sub><sup>TM</sup>

**Sizes contained:** HA<sub>10</sub>, HA<sub>12</sub>, HA<sub>14</sub>, HA<sub>16</sub>, HA<sub>18</sub>, HA<sub>20</sub>

NanoHA<sub>10-20</sub><sup>TM</sup> ladder is a preparation of different sizes of hyaluronic acid oligosaccharides, prepared by chemoenzymatic synthesis with immobilized enzyme reactors<sup>1</sup>. The reducing end contains *N*-acetylglucosamine. The amounts are adjusted for equal staining.

#### Quantity

The tube contains a mixture of six different oligo- or nanoHA oligomers supplied as a mixture of lyophilized ammonium and/or sodium salts. The material suffices to prepare 10 gels using the recommended procedures below.

#### Recommended Reconstitution Procedure

Centrifuge the tube for a few seconds to collect the nanoHA<sup>TM</sup> solids in the bottom of the tube. Carefully open and add 40  $\mu$ l of HPLC-grade water directly to the bottom of the tube. Mix well before use.

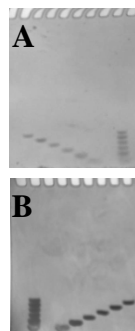
#### Electrophoresis Example

4  $\mu$ l of reconstituted sample with 1  $\mu$ l 2 M sucrose on an acrylamide gel results in clearly defined bands when stained with Alcian blue 8GX.<sup>2</sup>

The intensity of the bands can be increased by silver staining<sup>2</sup> (Fig. 1) and/or loading more sample.

#### Storage

For storage up to 1 week keep product at 4 °C. Otherwise freeze for long-term storage. Avoid contamination with microbes or HA-degrading enzymes.



From top to bottom:  
HA<sub>20</sub> 3811.2 Da  
HA<sub>18</sub> 3431.9 Da  
HA<sub>16</sub> 3052.6 Da  
HA<sub>14</sub> 2673.2 Da  
HA<sub>12</sub> 2293.9 Da  
HA<sub>10</sub> 1914.6 Da

**Figure 1. Electrophoretic analysis of nanoHA<sub>10-20</sub><sup>TM</sup>, lot no. nL080212**

**(A: Alcian Blue stained B: Silver stained)**

4  $\mu$ l reconstituted sample; 15 % Acrylamide/bis 29:1 (No stacking gel); Gel-buffer: 0.1 M Tris-Acetate, pH 7.0; Running buffer: 50 mM Tris Base/50 mM Tricine, pH 8.1-8.3; 200 V; 4 °C. (theoretical molecular masses are indicated).

#### References

- DeAngelis, P.L., Oatman, L.C., and Gay, D. F. (2003) Rapid chemoenzymatic synthesis of monodisperse oligosaccharides with immobilized enzyme reactors. *J. Biol. Chem.* **278**: 35199-35203.
- Ikegami-Kawai, M., Takahashi, T., (2002) Microanalysis of hyaluronan oligosaccharides by polyacrylamide gel electrophoresis and its application to assay of hyaluronidase activity. *Anal. Biochem.* **311**: 157-165.

**For research use only**

U.S. patent 6,444,447 and patents pending