

## APPENDIX

### APPENDIX A Preparation of some reagents used in determination of hyaluronan concentrations

#### 1. Coating buffer

$\text{NaHCO}_3$  4.2005 g

Dissolve chemical in 300 ml of distilled water. pH is adjusted to 9.6 and volume is added up to 500 ml.

#### 2. Citric phosphate buffer

Citric acid (monohydrate) 5.120 g

$\text{NaHPO}_4 \cdot 12\text{H}_2\text{O}$  18.265 g

All chemicals are dissolved in distilled water. pH is adjusted to 5 and volume is added up to 500 ml.

#### 3. Phosphate buffer saline (PBS)

$\text{NaCl}$  8 g

$\text{KCl}$  0.20 g

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  2.9 g

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  0.23 g

Dissolve all chemicals in distilled water and adjust pH to 7.4 with HCl. Then, add the volume up to 1 l with distilled water.

4. Phosphate buffer saline containing 0.01% Tween-20 (PBS-T)

Pipette 500  $\mu$ l of polyethylene sorbitan monolaurate (Tween-20) to a bottle of 1 l PBS, pH 7.4. Then, mix the solution thoroughly.



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**APPENDIX B** Evaluation of precision of the determination of hyaluronan level

The intra- and inter-assay precision were studied in the pooled human serum sample. Intra-assay was determined as 20 identical aliquots of pooled serum samples within the same plate. Inter-assay was determined in separate plates and performed with the same condition. The coefficient of variation was 14.66% and 15.33% for intra- and inter-assay, respectively. The results were shown below:

Assay	Hyaluronan Concentration (ng/ml±SD)	Coefficient of Variation (%)
Intra-assay	2,756.54±404.21	14.66
Inter-assay	2,698.90±413.83	15.33

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