Select-HA LADDERS™

Product Name	Catalog Number	Molecular Mass	Size
Select-HA™ MegaLadder	HYA-MGLAD	2 MDa – 9 MDa*	20 lanes
Select-HA™ HiLadder	HYA-HILAD	500 kDa – 1500 kDa*	20 lanes
Select-HA™ LoLadder	HYA-LOLAD	30 kDa – 500 kDa*	20 lanes

Support: echelon@echelon-inc.com

Description:
Molecular weight ladders for HA size determination.

Select-HA™ is a hyaluronic acid (HA) preparation of uniform and narrow size distribution prepared by in vitro synthesis using recombinant Pasteurella multocida hyaluronan synthase1.

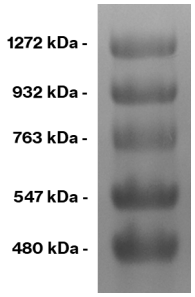
Select-HA™ HiLadder and LoLadder contain 5 Select-HA™ molecular mass markers while the Select-HA™ MegaLadder is a mixture of four biotin Select-HA complexes. Select-HA™ is a trademark of Hyalose LLC.

Properties:

Size – 20 lanes
Form – HiLadder and LoLadder: lyophilized (vial may appear empty), MegaLadder: solution of hyaluronan polymer sodium salts
Storage – -20 °C or below. Avoid frequent freeze-thaw, aliquoting is recommended. Avoid contamination with microbes or HA-degrading enzymes.
MW of ladders - Refer to the Certificate of Analysis (COA) for lot-specific molecular weights (M.W.) of ladder.

Background:
Hyaluronic acid (HA) is a high molecular weight anionic polysaccharide (1,000-10,000 kD) composed of repeating disaccharides and is one of several glycosaminoglycan components of the extracellular matrix of connective tissue. Various biological activities are influenced by the HA size or chain length including proliferation, angiogenesis, inflammation, and binding.

Data:
Example Agarose Gel
Refer to the Certificate of Analysis (COA) for lot-specific molecular weights (M.W.) of ladder.



Specific Information:

Electrophoresis of 5 µL reconstituted Select-HA Hi Ladder™ (lot# XCM090924) using a standard gel loading buffer on an an 0.75% agarose gel in TBE Buffer results in clearly defined bands when stained with 0.005% Stains-all (in 50% ethanol)[2].

Related Products:

Product	Catalog Number
Compounds	
BODIPY-HA	H-025F, H-250F, H-700F
Texas Red-HA	H-025R, H-250R, H-700R
Select-HA™	HYA-0050, HYA-0500, HYA-1000 (see website)
Biotinylated Select-HA™	HYA-B50-200, HYA-B250-200, HYA-B500-200, HYA-B1000-200
nanoHA™	HYA-NANO5-1
HAase Inhibitor	B-0601
HA Binidng Proteins	
Versican G1 Domain	G-HA01, G-HA02, G-HA03
Assays	
HA Quantification ELISAs	K-1200, K-4800, K-5800
Hyaluronidase Activity ELISA	K-6000

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Protocol for Hyaluronic Acid Gel Electrophoresis

This protocol is developed as a general guideline for hyaluronic acid (HA) molecular weight determination by gel electrophoresis. We recommend using this guideline as a starting point.

Materials:

- Hyaluronic Acid Samples to be tested (see HA Sample Preparation for more information)
- Hyaluronic Acid Standards (Echelon Select-HA™ Ladder, Cat. No. HYA-LOLAD-20, HYA-HILAD-20, HYA-MGLAD-20)
- High quality agarose or pre-poured polyacrylamide gel
- 1X Tris-Borate-EDTA (TBE) Buffer or 1X Tris-Acetate-EDTA (TAE) Buffer
- 1X Loading Buffer – 0.02% Bromophenol blue, 2 M sucrose in 1X TBE
- Stains-All Solution – 0.005% Stains-All in 50% ethanol, (Protect from light. Stains-All is light sensitive)
- Destaining Solution – 30% Ethanol
- Ultrapure Water

Protocol:

1. Reconstitute ladder if needed.
   - Reconstitution of HiLadder and LoLadder: Centrifuge the tube for a few seconds to collect the Select-HA™ solids in the bottom of the tube. Carefully open and add 100 µL of sterile water directly to the bottom of the tube. Allow two hours at 4 °C for sample hydration. Mix well before use.
   - The MegaLadder requires no reconstitution.
2. Prepare agarose solution in an Erlenmeyer flask with the selected buffer system. Refer to Table 1 for the recommended agarose concentration, gel dimension and buffer system.
3. Melt the agarose in a microwave oven, avoid overheating. Stop heating once the agarose solution is clear, then cool to a touchable temperature.
4. Pour the agarose solution into the gel-casting system. Insert comb and allow the gel to set. Once set, cover the gel with the selected buffer and let it sit overnight at room temperature.
5. If necessary, dilute HA samples with ultrapure water. Mix HA samples or Select-HA Ladder with 1X Loading Buffer at a 4:1 ratio. For example, mix 8 µL sample with 2 µL 1X Loading Buffer. Refer to Table 1 for recommended amounts of HA samples and HA standards.
6. Carefully remove comb from the gel, then place the gel into the electrophoresis apparatus of choice. Pour the selected buffer until the gel is fully covered.
7. Load the prepared HA samples or Select-HA standards (step 5) into the gel. Refer to Table 1 for loading concentration & volume.
8. Secure the electrophoresis unit cover. Confirm that the negative electrode is connected to the top of the gel and the positive electrode to the bottom.
9. Run gel for the designated time and voltage. Refer to Table 1 for recommended settings.
10. Immediately remove gel once electrophoresis is completed. Transfer the gel into a pre-wetted glass container with Stains-All Solution to prevent gel sticking.
11. Pour enough Stains-All Solution to cover the gel. Cover the glass container with lid or plastic wrap. Stain gel overnight at room temperature. Protect the glass container from light by wrapping the entire glass container with aluminum foil.
12. Carefully remove the Stains-All Solution.
13. Pour enough Destaining Solution to cover the gel. Cover the glass container with lid or plastic wrap. Destain the gel at room temperature for minimum overnight. Protect the glass container from light by wrapping the entire

Table 1. Recommend Gel Electrophoresis Condition for HA MW Determination[3-4]

HA MW	Gel/Buffer	Gel Dimension	Chamber Dimension	HA Sample Amount	Select-HA Ladder	Voltage & Run Time
>1500 kDa	0.5% Agarose in TAE	10 cm L x 6.2 cm W x 6.5 cm H	20 cm L x 15 cm W	3-10 µg/lane	5 µL/lane	20V for 30 minutes follow by 40V for 3.5 hours
100-1500 kDa	0.5-1% Agarose in TBE	10 cm L x 6.2 cm W x 6.5 cm H	25.5 cm L x 9.2 cm W	≤10 µg/lane	5 µL/lane	20V for 30 minutes follow by 40V for 3.5 hours
30-1000 kDa	1.5-2% Agarose in TBE	10 cm L x 6.2 cm W x 6.5 cm H	25.5 cm L x 9.2 cm W	≤10 µg/lane	5 µL/lane	Pre-electrophoresis at 40V for 20 minutes. Then, 20V for 30 minutes follow by 40V for 4 hours
10-500 kDa	3-4% Agarose in TBE	10 cm L x 6.2 cm W x 6.5 cm H	25.5 cm L x 9.2 cm W	≤10 µg/lane	5 µL/lane	Pre-electrophoresis at 40V for 20 minutes. Then, 20V for 30 minutes follow by 40V for 4 hours
4-100 kDa	4-20% Polyacrylamide in TBE	Pre-Cast	Invitrogen Xcell SureLock Mini Cell System	0.3-1 µg/lane	2-3 µL/lane	400 V for 28-40 minutes

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glass container with aluminum foil. Change Destaining Solution at least once to facilitate the destaining process.

14. When gel background is reduced, and bands are clear. Scan or image gel for record. Gel can be stored in the dark in the Destaining Solution for several days.
15. Discard the gel, Stains-All Solution and Destaining Solution as biohazard waste.

Frequent Ask Questions (FAQ):

1. Why is the Select-HA Ladder not clearly visible on the gel? This issue may be due to suboptimal gel running conditions. See Table 1.
2. Why are there no Select-HA bands on the gel? This is likely due to over-destaining without protecting the gel from light. Stains-All (CAS# 7423-31-6), used for HA visualization, is light-sensitive.
3. Can HA be visualized with stains other than Stains-All? Yes. Toluidin Blue has been used successfully (Andrade 2018), but Coomassie, does not stain HA.
4. How should I prepare samples for HA gel electrophoresis? HA must be extracted before analysis. Please see the HA Sample Preparation section in our HA Size Determination Protocol for details.
5. What is the shelf-life of the Select-HA Ladder? The Lo and Hi Ladders are lyophilized and remain stable for years at –20 °C. After reconstitution using sterile techniques, they remain stable for at least a year. The Mega Ladder is provided in solution and maintains stability for a year with sterile handling. For extended shelf life, aliquot into single-use portions.
6. What kind of gel is used in HA gel electrophoresis? Both agarose and polyacrylamide gels can be used, depending on the desired resolution and HA size range. See Table 1.
7. What loading buffer is used in HA gel electrophoresis? We recommend a loading buffer containing 0.02% bromophenol blue and 2 M sucrose in 1X TBE.
8. Can the Select-HA Ladder be substituted with a DNA or protein ladder? No. HA is a highly negative charged polysaccharide (a linear glycosaminoglycan) and migrates differently than DNA or protein on a gel.
9. How many µg HA is in each vial of Select-HA Ladder? The µg amount of HA per vial of ladder is lot specific and proprietary.
10. Why does the Select-HA Lo and Hi Ladder vial appear empty? The Select-HA Lo and Hi Ladders are supplied in lyophilized form, which may look empty. Reconstitution is required before use. *No reconstitution is required for the MegaLadder.*
11. Why is low voltage used in HA gel electrophoresis? Low voltage helps maintain HA band resolution and integrity. High voltage can lead to smearing and distorted bands.

Trouble shooting guide:

Issue	Possible reasons
Ladder is not visible on gel	• Was the gel over-destained? See FAQ 2 • Was the gel exposed to light during staining? See FAQ 2 • Was the correct gel used for ladder selected? See FAQ 1 and 6 • Was the correct stain used? See FAQ 3 • Was the ladder contaminated? See FAQ 5 • Was the correct amount of ladder added?
Smearing or distorted band	• Was the correct voltage used? See FAQ 11 • Was the ladder Overloaded?

References:

1. Jing, W.; DeAngelis, P. L. (2004) Synchronized chemoenzymatic synthesis of monodisperse hyaluronan polymers. J Biol Chem, 279 (40), 42345-9.
2. Lee, H. G.; Cowman, M. K. (1994) An agarose gel electrophoretic method for analysis of hyaluronan molecular weight distribution. Anal Biochem, 219 (2), 278-87.
3. Bhilocha, S.; Amin, R.; Pandya, M.; Yuan, H.; Tank, M.; LoBello, J.; Shytuhina, A.; Wang, W.; Wisniewski, H. G.; de la Motte, C.; Cowman, M. K., Agarose and polyacrylamide gel electrophoresis methods for molecular mass analysis of 5- to 500-kDa hyaluronan. Anal Biochem 2011, 417 (1), 41-9.
4. Cowman, M. K.; Chen, C. C.; Pandya, M.; Yuan, H.; Ramkishun, D.; LoBello, J.; Bhilocha, S.; Russell-Puleri, S.; Skendaj, E.; Mijovic, J.; Jing, W., Improved agarose gel electrophoresis method and molecular mass calculation for high molecular mass hyaluronan. Anal Biochem 2011, 417 (1), 50-6.

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