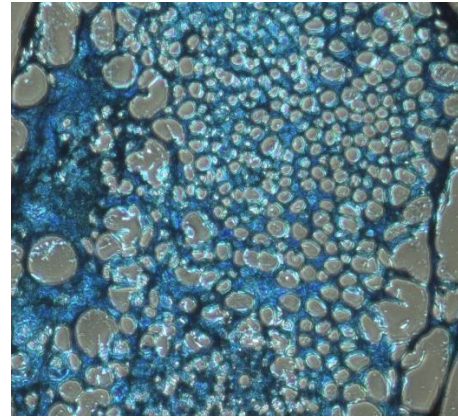


Alcian Blue Staining Kit Specifications and Instruction Quick Steps



Lifeline's adipose-derived mesenchymal stem cells differentiated to chondrocytes and stained with Alcian Blue (100X).

Alcian Blue Staining Kit Components

Alcian Blue stains sulfated proteoglycans deposits that are indicative of functional chondrocytes and it is a useful tool when used with Lifeline's mesenchymal stem cells and Lifeline's ChondroLife™ Complete Chondrogenesis Medium. This kit contains fixative solution, sucrose stabilizer solution, Alcian Blue stain solution, and acetic acid wash solution for staining sulfated proteoglycans deposited by chondrocytes.

Safety Statement

These products are for Research Use Only. These products are not approved for human or veterinary use, or for use in *in vitro* diagnostics or clinical procedures. Please use and dispose of paraformaldehyde, and reagents in accordance with local and federal regulations.

The Lifeline Guarantee

Lifeline's rigorous quality control ensures sterility and performance to standardized testing criteria. Upon request, Lifeline will provide lot-specific QC test results, material safety data sheets and certificates of analysis. See complete guarantee/warranty statement at lifelinecelltech.com or contact your Lifeline representative for more information.

Product	Part No.	Volume	Storage
Alcian Blue Staining Kit	LL-0051		
20% Sucrose Stabilizer	CM-0051	10 mL	Room temp.
1% Alcian Blue Stain Solution	CM-0052	100 mL	Room temp.
3% Acetic Acid Wash	CM-0053	100 mL	Room temp.
4% Paraformaldehyde Fixative Solution	CM-0055	100 mL	-20°C
Additional Materials Recommended but Not Provided by Lifeline Cell Technology			
<ul style="list-style-type: none"> • Embedding compound (such as OCT™ Compound) • Cryomolds • Microtome 		<ul style="list-style-type: none"> • Absolute ethanol • Dry ice/isopropanol bath • Glass slides 	

(OCT is a trademark of Tissue-Tek®, Andwin Scientific)

To place an order, please visit lifelinecelltech.com or call customer service at 877.845.7787.

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**Quick Steps for Fixation and Staining of Sulfated Proteoglycan Deposits in Chondrogenic Microbeads
(Cultured in a 48-Well Plate Format)**

1. **Always wash hands before and after working with cell cultures.**
2. **Always wear eye protection and gloves when working with cell cultures.**
3. **When working with cells or medium, always use a certified biological safety cabinet.**
4. **Use 4% Paraformaldehyde Fixative Solution in a chemical fume hood.**
5. Remove medium completely from well(s).
6. Add 0.5 mL 4% Paraformaldehyde Fixative Solution (CM-0055) per well.
7. Fix Microbeads for 3 hours at Room Temperature. Shield from light.
8. Remove fixative and add 0.5 mL of 20% Sucrose Stabilizer (CM-0051) per well.
9. Incubate overnight at room temperature. Shield from light.
10. Transfer Chondrogenic Microbeads to a plastic cryomold to embed them in a suitable material (such as OCT™ Compound, or equivalent) for slicing on a microtome or cryostat.
11. Remove residual sucrose solution.
12. Gently layer the embedding material over the Chondrogenic Microbeads so as to maintain separation of the individual Microbeads and maintain their location towards the bottom of the cryomold.
13. Snap freeze the cryomold in a dry ice/isopropanol bath. Store at -80°C until ready to cut.
14. Cut 5 µm sections from block and mount on glass slide.
15. Place slide in 3% Acetic Acid Wash (CM-0053) for 3 minutes.
16. Place in 1% Alcian Blue Stain Solution (CM-0052) for 30 minutes.
17. Wash in running tap water for 1 minute.
18. Dip briefly in absolute ethanol to dehydrate and air dry.
19. Staining is complete.

For any question on cell staining, please contact technical service at 877.845.7787. We are here to help.

Other Recommended Products	Part No.	Unit	Components	Storage
Human Mesenchymal Stem Cells-Wharton's Jelly (HMSC-WJ)	FC-0020	0.5 x 10 ⁶ Cells		-150°C
Human Mesenchymal Stem Cells-Adipose (HMSC-Ad)	FC-0034	10 ⁶ Cells		-150°C
StemLife™ MSC Medium Complete Kit	LL-0034	Kit	StemLife Basal Medium StemLife MSC LifeFactors® Kit	2-8°C when prepared
Human Mesenchymal Stem Cells-Bone Marrow (HMSC-BM)	FC-0057	10 ⁶ Cells		-150°C
StemLife MSC-BM Medium Complete Kit	LL-0062	Kit	StemLife Basal Medium StemLife MSC-BM LifeFactors Kit	2-8°C when prepared
Human Pre-Adipocytes, Adult (HMSC-Pre-Adipocyte)	FC-0062	10 ⁶ Cells		-150°C
StemLife PA Medium Complete Kit	LL-0058	Kit	StemLife PA Basal Medium StemLife PA LifeFactors Kit	2-8°C when prepared
ChondroLife™ Complete Chondrogenesis Medium	LM-0022	100 mL		-20°C
TrypKit™	LL-0013	Kit	PBS 0.05% Trypsin/0.02% EDTA Trypsin Neutralizing Solution	RT -20°C -20°C
Optional Supplements	Part No.	Volume	Concentrations of Supplement	Storage
Phenol Red Supplement	LS-1009	1 mL	33 mM	RT
Antimicrobial Supplement: Gentamicin and Amphotericin B (Provided with purchase of LL-0034, LL-0058, or LL-0062)	LS-1104	0.5 mL	Gentamicin 30 mg/mL Amphotericin B 15 µg/mL	-20°C

Call Lifeline Technical Service and Sales at 877.845.7787
or visit lifelinecelltech.com for more information

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