

Materials List for:

Using a GFP-tagged TMEM184A Construct for Confirmation of Heparin Receptor Identity

Sara Lynn N. Farwell¹, Joshua B. Slee², Yaqiu Li¹, Linda J. Lowe-Krentz¹

¹Department of Biological Sciences, Lehigh University

²Department of Natural Science, DeSales University

Correspondence to: Linda J. Lowe-Krentz at ljlo@lehigh.edu

URL: <https://www.jove.com/video/55053>

DOI: [doi:10.3791/55053](https://doi.org/10.3791/55053)

Materials

Name	Company	Catalog Number	Comments
GFP-TMEM184A construct	OriGene	RG213192	
Rhodamine-Heparin	Creative PEGWorks	HP-204	Light Sensitive
Fluorescein-Heparin	Creative PEGWorks	HP-201	Light Sensitive
Mowiol	EMD Millipore	475904-100GM	
Paraformaldehyde (PFA, methanol free)	Thermo Sci Pierce Biotech, available through Fisher Scientific	PI28908 at Fisher	Use in Fume Hood
Reacti-bind neutravidin plates (Avidin coated black 96-well dishes)	Thermo Sci Pierce Biotech, through Fisher Scientific	PI15510 at Fisher	Pay attention to shelf-life
Black 96-well plates	Corning Life Sciences Plastic, purchased through Fisher Scientific	064432 at Fisher	
A7r5 vascular smooth muscle cell line	ATCC	CRL 1444	Can be exchanged into MEM medium ¹
BAOEC bovine aortic endothelial cells	Cell Applications, Inc.	B304-05	Culture as recommended initially, can be exchanged into MEM medium for continuing culture ^{1,7}
BAOSMC bovine aortic smooth muscle cells	Cell Applications, Inc.	B354-05	Culture as recommended initially, can be exchanged into MEM medium for continuing culture ¹
RAOEC rat aortic endothelial cells	Cell Applications, Inc.	R304-05a	Culture as recommended initially, can be exchanged into MEM medium for continuing culture ⁷
Biotinylated anti-GFP	Thermo Sci Pierce Biotech, through Fisher Scientific	MA5-15256-BTIN	
Streptavidin-coated beads	Sigma	S1638	
HeBS	Available from Bio-Rad		Can be prepared in the lab. The pH is 6.8
TMEM184A antibody to the N-terminus	Santa Cruz Biotechnology	sc292006	Only known TMEM184A antibody to N-terminal region.
TMEM184A antibody to the C-terminus	Obtained from ProSci Inc, Poway, CA	Pro Sci 5681	ProSci used in Figure 1
GFP antibodies	Santa Cruz Biotechnology	sc9996	Used in Figure 5
Secondary antibodies, labeled with TRITC or Cy3	Jackson ImmunoResearch Laboratories, Inc, West Grove, PA	711 025 152 (donkey anti-rabbit, TRITC) 715 165 150 (donkey anti-mouse, Cy3)	Minimal cross-reactivity to minimize any nonspecific staining.
CHAPS	Purchased from Sigma	C5849	Note that this specific catalog number has been discontinued. Supplier will provide information regarding replacement.

Live imaging 35-mm dishes	MatTek (Ashland MA)	P35G-1.0 – 20 mm - C	
Confocal Microscope	Zeiss	LSM 510 Meta with a 63X oil-immersion lens	Used for images and live-imaging in Figures 1, 2 and 3
Confocal Microscope	Nikon	C2+ confocal with a 60X oil-immersion lens	Used for images in Figure 5
Confocal Microscope	Zeiss	Zeiss LSM 880 with a 63X oil-immersion lens	Used for images in Figure 2C
Electroporation equipment	Bio-Rad	Gene Pulser X-Cell System	
Electroporation cuvettes	Available from MidSci	EC2L	Can also be obtained from equipment supplier
Plate reader	TECAN	TECAN Infinite® m200 Pro plate reader	Readings in the middle of the wells rather than at the surface.
Computer program for measuring staining intensity	Image J	https://imagej.nih.gov/ij/ Program and information available on-line	Any appropriate program can be used. See https://theolb.readthedocs.io/en/latest/imaging/measuring-cell-fluorescence-using-imagej.html for additional detail
Cell Culture trypsin solution	Sigma	T4174	purchased as a 10x solution