Applied Biosystems® Arcturus® HistoGene® LCM Immunofluorescence Staining Kit

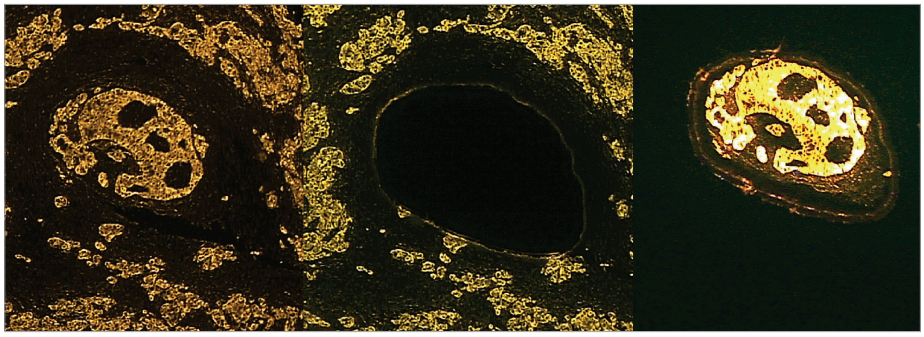

Figure 1. Target cell after treatment with the HistoGene® LCM Immunofluorescence Staining Kit highlights specific surface and intracellular proteins.

Benefits

- Quick 15-minute procedure preserves RNA integrity
- Simple fluorescent target cell labeling
- High-contrast label intensity
- Reduced background fluorescence and stable labeling

Immunofluorescently Stain Frozen Tissue Sections While Preserving RNA

Antigen markers that highlight specific surface or intracellular proteins enable scientists to identify target cells for laser capture microdissection (LCM) and RNA expression analysis (Figure 1). The Applied Biosystems® Arcturus® HistoGene® LCM Immunofluorescence Staining Kit from Life Technologies is specifically designed for retrieval of high-quality RNA from immunofluorescently stained, frozen tissue sections (Figure 2). The staining kit provides reagents and slides for convenient and reliable immunofluorescent staining, and includes a protocol—streamlined and optimized to maintain RNA quality—and materials sufficient for processing 32 slides.

Quick Process to Preserve RNA

Standard immunofluorescence labeling kits generally require more than 90 minutes to process samples—suboptimal conditions for single-stranded nucleic acid stability that compromises RNA integrity. In contrast, the HistoGene® LCM Immunofluorescence Staining Kit's protocol processes samples typically in less than 15 minutes (only 5 minutes in an aqueous environment) and uses a proprietary staining buffer that minimizes RNA degradation. Electrophoresis and RT-PCR of isolated RNA confirms that the HistoGene® LCM Immunofluorescence Staining Kit retrieves high-quality RNA from microdissected samples.

Chill Samples for Intact RNA

The HistoGene® Cold Block increases RNA yield and helps ensure its quality by keeping up to four tissue-section slides and several tubes containing buffer and antibody solutions chilled during staining kit processing (Figure 3). The block is designed for use with the CoolSafe triple-density polystyrene cooler and the –10°C CoolBrick from Diversified Biotech (www.divbio.com).

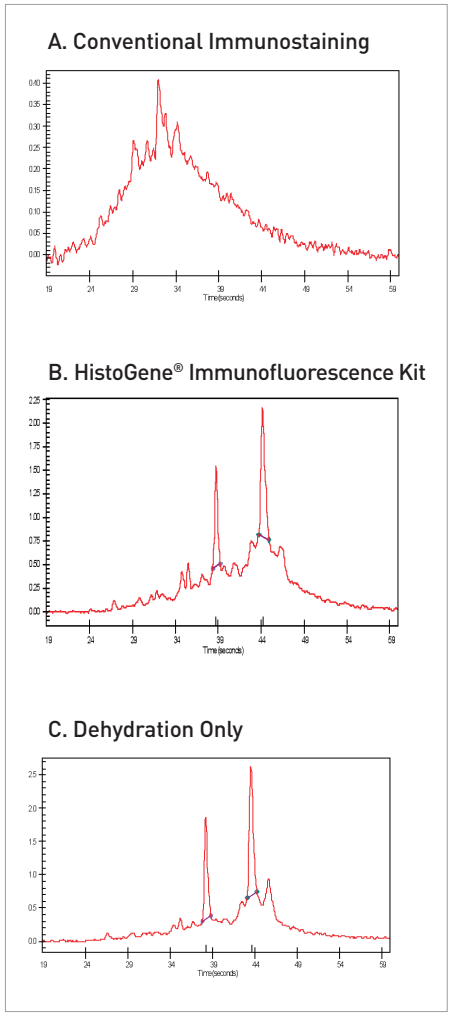

Figure 2. HistoGene® LCM Immunofluorescence Staining Kit demonstrates superior data. Bioanalyzer profiles showing RNA quality from a sample stained using **(A)** conventional immunofluorescence staining procedures and **(B)** the HistoGene® Immunofluorescence procedure, as compared with **(C)** the control, which was dehydrated. The data show that the HistoGene® Immunofluorescence Kit gives clear data with high-quality RNA results.

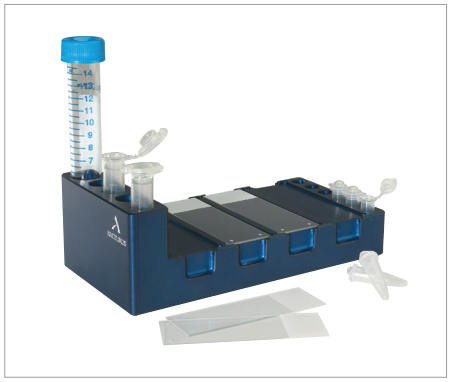

Figure 3. The HistoGene® Cold Block.

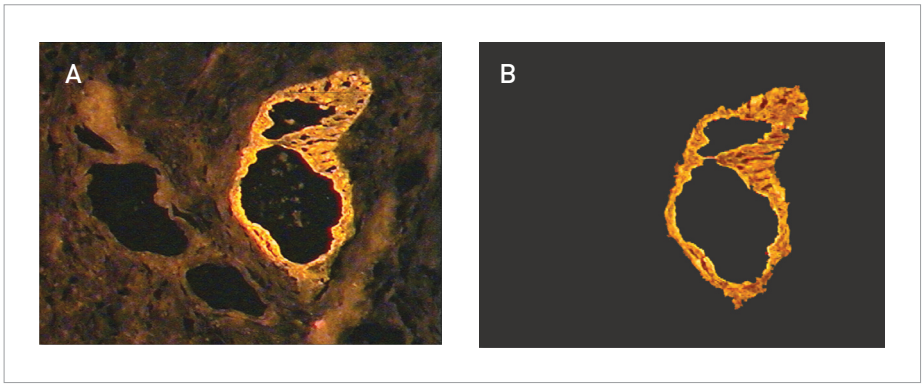

Figure 4. HistoGene® LCM Immunofluorescence Staining Kit laser capture microdissection of human prostate. Laser capture microdissection (LCM) of human prostate ductal epithelium prepared with the HistoGene® LCM Immunofluorescence Kits. **(A)** Human prostate tissue stained with an anti-cytokeratin antibody, before LCM. **(B)** Image of the captured cytokeratin positive cells on the cap after LCM.

Brilliant High-Contrast Label Intensity

The HistoGene® LCM Immunofluorescence Staining Kit employs a biotin–avidin system with Cy®3 dyes, resulting in exceptionally good staining intensity and specificity (Figure 4). The staining kit is used with a primary biotinylated monoclonal antibody to an antigen of choice provided by the user, and Cy®3 conjugated streptavidin is included in the kit (Figures 4 and 5).

Use Microgenomics Products for Microarray Analysis or qPCR

The HistoGene® LCM Immunofluorescence Staining Kit is part of Life Technologies' complete System for Microgenomics®—products designed to seamlessly work together to produce high-quality expression microarray data from pure cell populations.

Use HistoGene® kit–stained samples with the Applied Biosystems® Arcturus$^{XT™}$ LCM System to capture pure cell populations of fluorescently labeled target cells. Maximize recovery of RNA from even small numbers of cells with the PicoPure® RNA Isolation Kit. Next, amplify nanogram quantities of RNA to micrograms using the RiboAmp® RNA Amplification Kit, which provides amplified antisense RNA ready for labeling and hybridization to microarrays (Figure 6).

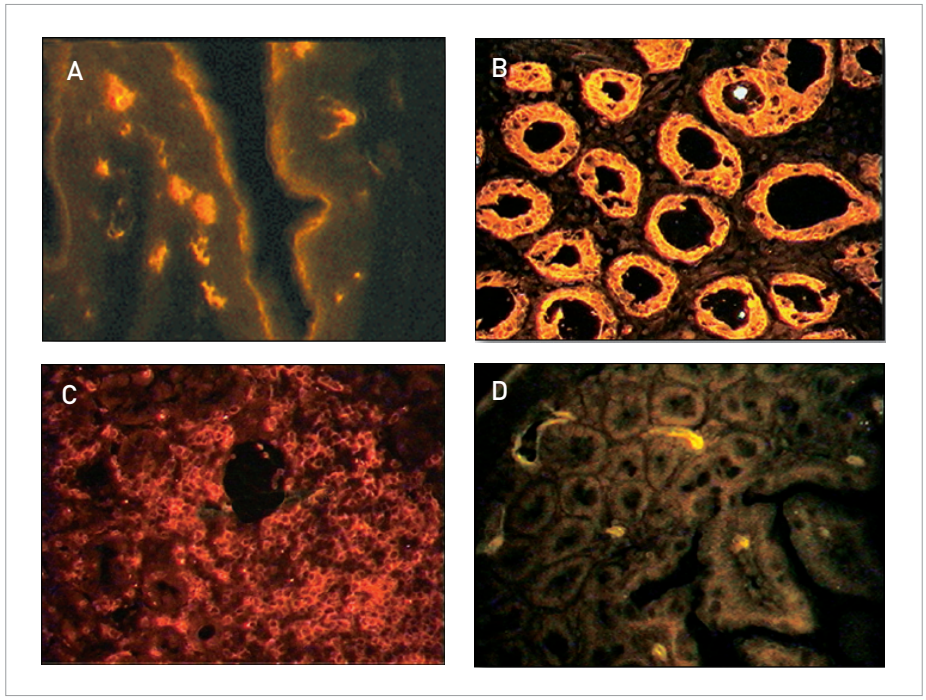

Figure 5. Identify target cells using the HistoGene® LCM Immunofluorescence Staining Kit. (A) Human foreskin tissue stained with anti-CD1a antibody. **(B)** Human jejunum tissue stained with anti-Pan-cytokeratin antibody. **(C)** Mouse lacrimal gland inflammatory infiltrate stained with anti-CD4 antibody. **(D)** Mouse small intestine tissue stained with anti-MAdCAM-1 antibody.

Obtain Intact RNA from Many Tissue Types

After using the HistoGene® LCM Immunofluorescence Staining Kit on many tissue types with several antibodies, scientists examined RNA integrity. All tissue types tested using the HistoGene® Immunofluorescence Kits yielded high-quality RNA (Figure 7).

Obtain High-Quality RNA from Many Tissue Types

The HistoGene® LCM Immunofluorescence Staining Kit has been validated by examining RNA integrity on an expanding list of tissue types. All tissues tested to date using this kit have yielded quality RNA (Table 1).

Table 1. Validated tissue-antibody sets.

Validated Tissue-Antibody Sets	
Tissue	**Antibody**
Human skin	CD1a
Human jejunum	Pan-cytokeratin
Human breast	Progesterone receptor, Pancytokeratin, Her2-neu, Estrogen R
Human prostate	Pan-cytokeratin
Mouse spleen	CD4
Mouse lacrimal	Gland CD4, CD 45
Mouse brain	GFAP
Mouse small	Intestine CD4, MAdCAM-1
Mouse thymus	CD4

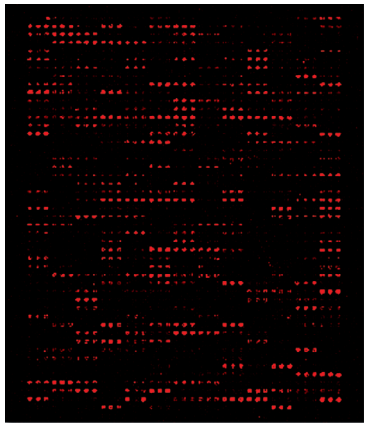

Figure 6. LCM sample expression microarray. LCM was used to collect samples, which were prepared using HistoGene® LCM Immunofluorescence, PicoPure® RNA Isolation, and RiboAmp® RNA Amplification Kits. Human breast tumor tissue sections were stained for cytokeratin using the HistoGene® LCM Immunofluorescence Kit, and 1,000 positively stained cells were microdissected. RNA was isolated using the PicoPure® RNA Isolation Kit and amplified with the RiboAmp® RNA Amplification Kit. Amplified RNA was converted to Cy®5 dye–labeled cDNA, hybridized to a 24,000 element human cDNA array, and scanned.

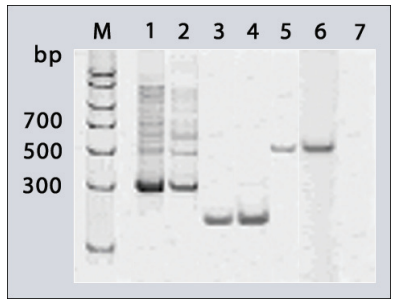

Figure 7. Detection of low- and high-abundance, full-length RNA. RT-PCR was performed on RNA from 500–1,000 cells captured from different tissues. Equal quantities of cDNA were analyzed with primers detecting 3′ and 5′ ends of low-abundance ADP ribosylation factor 1 (ARF-F1), (3′, 239 bp; 5′, 336 bp) and the 5′ end of the clathrin gene, which has a transcript length over 6 kb (570 bp) (primer sets from KPL, Inc.). M: molecular-weight markers. Lane 1: human breast tissue, ARF-F1, 5′. Lane 2: mouse brain, ARF-F1, 5′. Lane 3: mouse spleen, ARF-F1, 3′. Lane 4: mouse lacrimal gland, ARF-F1, 3′. Lane 5: mouse lacrimal gland, clathrin. Lane 6: human prostate, clathrin. Lane 7: negative RT control, human prostate, clathrin.

ORDERING INFORMATION

Description	Size	Part Number
HistoGene® LCM Immunofluorescence Staining Kit	Contains reagents for staining 32 slides HistoGene® Buffer A (4 mL) HistoGene® Buffer B (60 mL) HistoGene® Cy®3 streptavidin (60 µL) 72 HistoGene® LCM immunofluorescence staining slides User guide	KIT0420
HistoGene® Cold Block	1 block	HIS0101
Related Products		
HistoGene® LCM Frozen Section Staining Kit	Contains reagents for processing 72 slides	KIT0401
RiboAmp® RNA Amplification Kit	Contains reagents for 10 amplifications	KIT0521
RiboAmp® OA RNA Amplification Kit	Contains reagents for 10 amplifications	KIT0208
RiboAmp® HS RNA Amplification Kit	Contains reagents for 5 two-round amplifications	KIT0525
PicoPure® RNA Isolation Kit	Contains reagents for 40 isolations	KIT0204

For additional information on the HistoGene® LCM Immunofluorescence Staining Kit and to place your order, visit www.appliedbiosystems.com/arcturus.

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applied biosystems by life technologies

Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.327.3002
www.appliedbiosystems.com

International Sales
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