

HistoGene LCM Immunofluorescence Staining Kit

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™ HistoGene™ LCM Immunofluorescence Staining Kit 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, which compromise RNA integrity. In contrast, the HistoGene LCM Immunofluorescence Staining Kit’s protocol describes how to process samples, typically in less than 15 minutes (only 5 minutes in an aqueous environment), using 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.

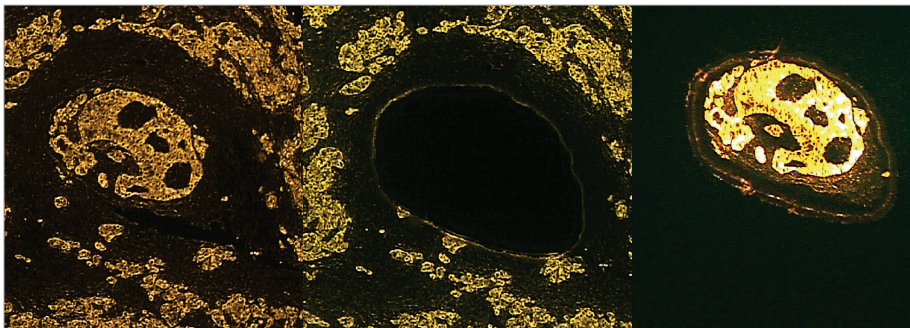


Figure 1. Target cell after treatment with the HistoGene LCM Immunofluorescence Staining Kit highlights specific surface and intracellular proteins.

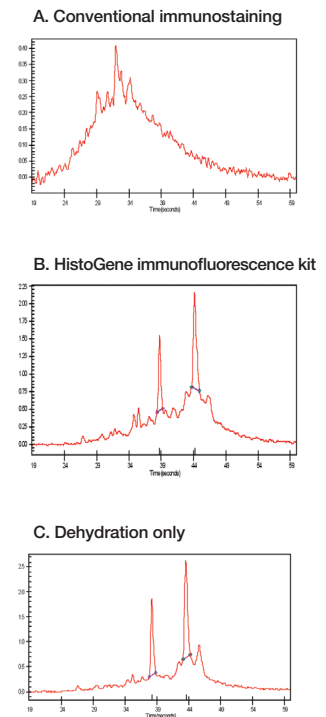


Figure 2. The HistoGene LCM Immunofluorescence Staining Kit enables recovery of high-quality RNA. Bioanalyzer profiles showing RNA quality from a sample stained using (A) a conventional immunofluorescence staining procedure and (B) the HistoGene immunofluorescence procedure, as compared with (C) the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

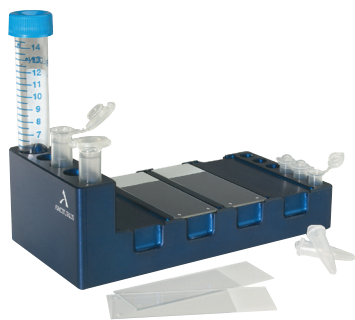


Figure 3. The HistoGene Cold Block.

Chill samples for intact RNA

The Applied Biosystems™ HistoGene™ Cold Block enables increased RNA yield and helps ensure RNA 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 Cool Brick from Diversified Biotech (divbio.com).

Brilliant high-contrast label

The HistoGene LCM-a Immunofluorescence Staining Kit employs a biotin-avidin system with Cy³ dyes, resulting in exceptionally good staining intensity and specificity (Figure 4). A primary biotinylated monoclonal antibody to an antigen of choice is provided by the user, and a Cy3 dye-streptavidin conjugate 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 our complete Applied Biosystems™ Systems for Microgenomics™ line of products designed to seamlessly work together to produce high-quality expression microarray data from pure cell populations.

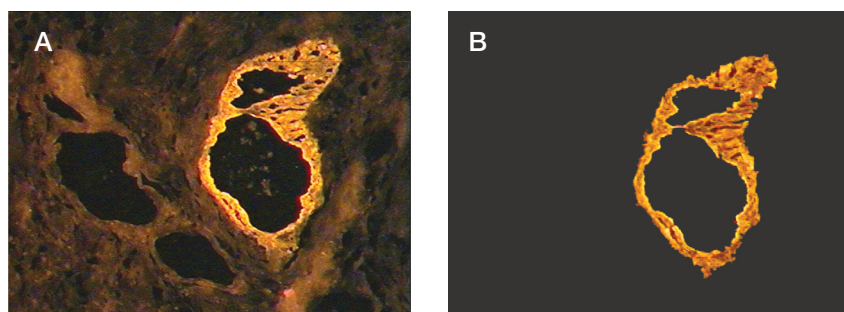


Figure 4. LCM of human prostate ductal epithelium prepared with the HistoGene LCM Immunofluorescence Staining Kit. (A) Human prostate tissue stained with an anti-cytokeratin antibody, before LCM. **(B)** Image of the captured cytokeratin-positive cells on the cap after LCM.

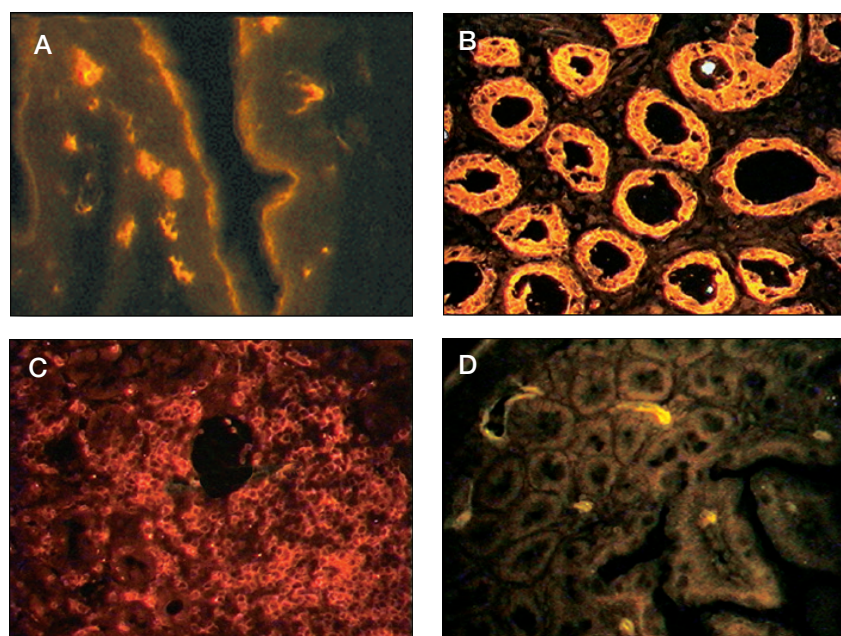


Figure 5. Identify target cells using the HistoGene LCM Immunofluorescence Staining Kit. (A) Human foreskin tissue stained with anti-CD1a antibody. **(B)** Human jejunum tissue stained with anti-pan-cytokeratin antibody. **(C)** Mouse lacrimal gland inflammatory infiltrate stained with anti-CD4 antibody.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

Use HistoGene kit-stained samples with the Applied Biosystems™ ArcturusXT™ LCM System to capture pure cell populations of fluorescently labeled target cells. Maximize recovery of RNA from even small numbers of cells with the Applied Biosystems™ PicoPure™ RNA Isolation Kit. Next, amplify nanogram quantities of RNA to micrograms using the Applied Biosystems™ RiboAmp™ RNA

Amplification Kit, which enables the production of amplified antisense RNA ready for labeling and hybridization to microarrays (Figure 6).

Obtain high-quality RNA from many tissue types

We have used the HistoGene LCM Immunofluorescence Staining Kit on a variety of tissue types with several antibodies and examined RNA integrity. All tissue types tested to date using the HistoGene LCM Immunofluorescence Staining Kit yielded high-quality, full-length RNA (Table 1 and Figure 7).

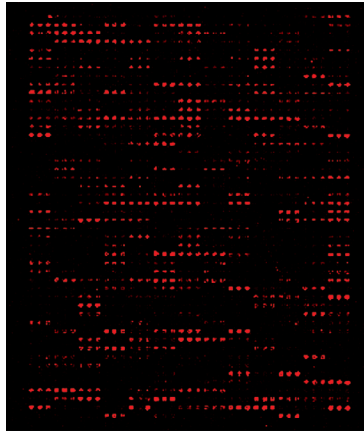


Figure 6. LCM sample expression microarray. Human breast tumor tissue sections were stained for cytokeratin using the HistoGene LCM Immunofluorescence Staining Kit, and 1,000 positively stained cells were collected by LCM. RNA was isolated using the PicoPure RNA Isolation Kit and amplified with the RiboAmp RNA Amplification Kit. Amplified RNA was converted to Cy5 dye-labeled cDNA, hybridized to a 24,000-element human cDNA array, and scanned.

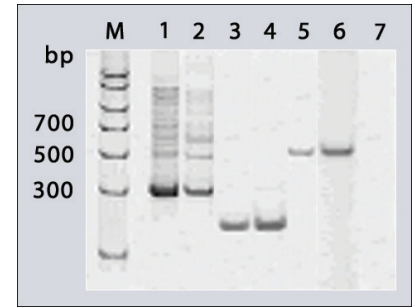


Figure 7. Detection of low- and high-abundance, full-length RNA. RT-PCR was performed on RNA from 500–1,000 cells captured from different tissues. Equal quantities of cDNA were analyzed with primers detecting 3' and 5' ends of low-abundance ADP ribosylation factor 1 (ARF-F1), (3', 239 bp; 5', 336 bp) 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.

Table 1. Validated tissue-antibody sets.

| Tissue | Antibody |
|-----------------------|---|
| Human skin | CD1a |
| Human jejunum | Pan-cytokeratin |
| Human breast | Progesterone receptor, pan-cytokeratin, Her2-neu, estrogen receptor |
| Human prostate | Pan-cytokeratin |
| Mouse spleen | CD4 |
| Mouse lacrimal gland | CD4, CD45 |
| Mouse brain | GFAP |
| Mouse small intestine | CD4, MAdCAM-1 |
| Mouse thymus | CD4 |

Ordering information

| Product | Quantity / contents | Cat. No. |
|---|---|----------|
| HistoGene LCM Immunofluorescence Staining Kit | Reagents for staining 32 slides HistoGene Buffer A (4 mL) HistoGene Buffer B (60 mL) HistoGene Cy3 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 | Reagents for processing 72 slides | KIT0401 |
| RiboAmp PLUS RNA Amplification Kit | Reagents for 12 one-round amplifications or 6 two-round amplifications | KIT0521 |
| RiboAmp HS PLUS RNA Amplification Kit | Reagents for 6 two-round amplifications | KIT0525 |
| PicoPure RNA Isolation Kit | Reagents for 40 isolations | KIT0204 |

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