Application Note

Histochemical Staining of Tissues with SOMAmer® Reagents

Introduction

SOMAmer reagents (Slow Off-rate Modified Aptamers) are DNA-based high affinity (average Kd < 1 nM) protein binding reagents with proprietary chemical modifications that provide hydrophobic characteristics not present in natural DNA. These modifications enhance protein binding through direct hydrophobic contacts with the target protein, resulting in increased binding affinities and slower complex dissociation rates compared to unmodified aptamers. This Application Note discusses the suitability of SOMAmer reagents in histochemical staining of tissues. **Please note that not all SOMAmer reagents have been tested in all applications.** For help or technical support, please submit your questions by either calling the U.S. Technical Support at 1-800-324-0783, emailing techsupport@somalogic.com or by clicking here to visit the technical support website.

Histochemical staining of tissues is used clinically to identify cell types by the presence or absence of marker proteins. Antibodies are currently the reagent of choice for this application, but slow diffusion of antibodies in tissues and the need for a secondary staining reagent or signal amplification limit their utility. SOMAmer reagents are approximately 10 times smaller than antibodies and can be directly labeled with a fluorescent dye to circumvent these limitations. Furthermore, the combined properties of high affinity, specificity, and slow off-rate make SOMAmer reagents valuable reagents for histochemistry applications.

Inclusion of a competitor during or after SOMAmer incubation can be valuable in distinguishing between specific and non-specific binding in all SOMAmer reagent applications. While many applications do not involve high levels of proteins with high random DNA affinity, they often include very high concentrations of proteins with modest affinity (e.g., some highly abundant plasma proteins that can bind DNA). Addition of a competitor can help reduce artifacts that may arise from random DNA binding layered on specific SOMAmer reagent binding. The SomaLogic® Polyanionic Competitor [Product Number 910-00001] or dextran sulfate at concentrations ranging from 0.1 µM to 10 mM should be evaluated for use in specific applications.

Protocol: Selective Staining of Her2+ Cells in Frozen Sections with Her2 SOMAmer Reagent

SOMAmer reagents that bind human Her2 were developed by SomaLogic using the SELEX process from a chemically modified DNA library. One Her2 SOMAmer reagent was labeled at its 5' terminus with a cyanine fluorescent dye and applied to frozen breast carcinoma tissue sections pre-classified as either Her2-positive or Her2-negative (Gupta et al., 2011). The polyanion dextran sulfate was co-applied to minimize nuclear staining. The Her2 SOMAmer reagent showed membrane staining of cells in the Her2-positive sections, and no staining of the Her2-negative sections (Figure 1). SOMAmer staining was rapid, saturating its target in less than 1 minute.

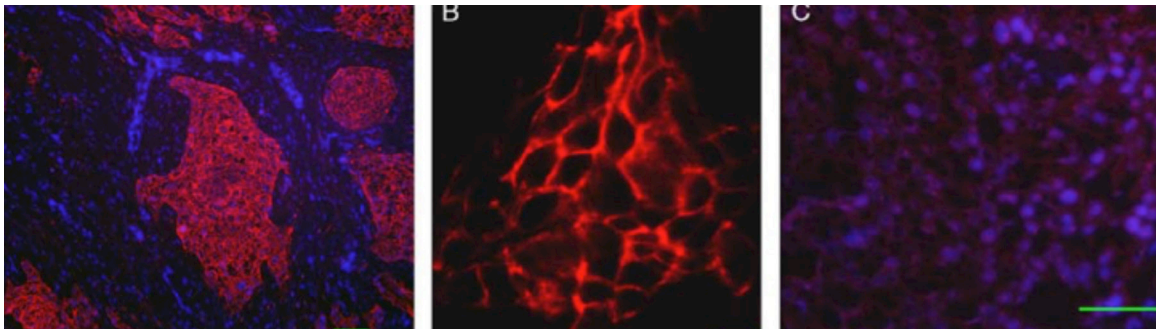

Figure 1. (A) 100 nM HER2 SOMAmer/1 mM dextran sulfate (DS) applied to HER2-positive tissue section from stage 3+ invasive ductal carcinoma sample, nuclei counterstained with DAPI. (B) cyanine-channel deconvolution microscopy image from identical sample as in (A), showing membranous SOMAmer localization. (C) 100 nM HER2 SOMAmer/1mM DS applied to HER2-negative tissue section from stage 0+ invasive ductal carcinoma, nuclei counterstained with DAPI. Images from Gupta et al. 2011.

Recommended Materials

- Binding Buffer (BB): 40 mM Na-HEPES, pH 7.5, 52 mM NaCl, 5 mM KCl, 5 mM MgCl2
- Dextran sulfate (8000 Da)
- SOMAmer reagent labeled with cyanine dye (alternatively, a biotin-conjugated SOMAmer reagent coupled to dye-labeled streptavidin may be used)
- Charged microscope slide
- Microscope with filters appropriate for imaging the fluorescent dye

Preparation of Tissue

1. Place 5 µm thick frozen tissue section onto charged slide
2. Immerse tissue in fixative solution (100% ethanol or acetone) for at least 1 hour
3. Rinse slide once in de-ionized water for 2 minutes and once in BB for 2-5 minutes

SOMAmer Staining of Tissue

1. Prepare 100 nM SOMAmer solution in BB + 1 mM dextran sulfate
2. Incubate SOMAmer solution 95°C for 10 minutes, slowly cool to room temperature
3. Apply SOMAmer solution to tissue for 10 minutes
4. Wash once with BB + 1 mM dextran sulfate for 5 minutes
5. Capture images with fluorescent microscope

References:

Gupta et al. 2011, Rapid Histochemistry Using Slow Off-rate Modified Aptamers with Anionic Competition, Appl. Immunohistochem. Mol. Morphol. 19:273

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