F-Actin Probes in Living Cells

Dynamic remodeling of the actin cytoskeleton (i.e., rapid cycling between filamentous actin (F-actin) and monomer actin [G-actin]) is required for multiple physiological functions, including intracellular transport, cell growth, morphology, motility, trafficking, polarity, cell-to-cell contacts, and cytokinesis\(^1\). Correspondingly, dysfunctional actin cytoskeletal dynamics are a pathophysiological feature of many human diseases, including those with oncogenic, neurodegenerative, or cardiovascular origins\(^2\). For these reasons, there is continuing interest in F-actin live cell imaging probes to study actin cytoskeletal dynamics in cell culture models of health and disease (Table 1).

**SiR and SPY Actin Probes**

The ideal actin visualization tool is a small molecule able to bind F-actin in a sensitive and selective manner, while not disrupting actin re-modeling. In addition, introduction directly into the cell culture medium or tissues without need for transfection or electroporation is advantageous\(^3,4\). The new SiR/SPY actin probes fulfill the needs of an “ideal” actin-binding molecule while surmounting most, if not all, of the concerns and shortcomings associated with existing actin probes (Table 1). Initially characterized by Lukinavicius et al.\(^5,6\) and introduced commercially in 2014, the SiR and SiR700-actin live cell imaging probes label endogenous F-actin and avoid the need for transfections and over-expression of labeled actin proteins or actin-binding proteins\(^7,8\). SiR/SiR700-actin probes are structurally related to the naturally occurring F-actin binding molecule jasplakinolide\(^9,10\). These F-actin probes utilize the proprietary fluorophore silicon rhodamine (SiR), a bright, photostable far-red dye with little, if any, background signal (Figs. 1,2). Because SiR probes exist in a closed, non-fluorescent state (spirolac tone), the probes are self-quenching when unbound to F-actin\(^11\). SiR probes are visualized with standard Cy5 settings (optimal excitation, 650 nm; emission, 670 nm) which confer compatibility with a wide range of genetically-encoded reporter fluorophores (e.g., GFP, m-Cherry)\(^12\).

**SPY555-actin** is the newest addition to Spirochrome’s family of F-actin live cell imaging probes. SPY555-actin is an improved encoded reporter fluorophore (e.g., GFP, m-Cherry)\(^12\). SPY555-actin is the newest addition to Spirochrome’s family of F-actin live cell imaging probes. SPY555-actin is an improved encoded reporter fluorophore (e.g., GFP, m-Cherry)\(^12\). SPY555-actin is the newest addition to Spirochrome’s family of F-actin live cell imaging probes. SPY555-actin is an improved encoded reporter fluorophore (e.g., GFP, m-Cherry)\(^12\). SPY555-actin is the newest addition to Spirochrome’s family of F-actin live cell imaging probes. SPY555-actin is an improved encoded reporter fluorophore (e.g., GFP, m-Cherry)\(^12\).

**Research Tools**

F-Actin Probes in Living Cells

Related Publications

Meetings

Cold Spring Harbor Conference - Systems Biology: Global Regulation of Gene Expression
March 11-14th

Cold Spring Harbor, NY
Cytoskeleton Supported

Cold Spring Harbor Conference - Neuronal Circuits
March 18-21st

Cold Spring Harbor, NY
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Cold Spring Harbor Conference -from Neuroscience to Artificially Intelligent Systems
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Activation Assays
Antibodies
ECM Proteins
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Pull-down Assays
Motor Proteins
Small G-Proteins
Tubulin & FtsZ Proteins

Contact Us
P: 1 (303) 322.2254
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E: cserve@cytoskeleton.com
W: cytoskeleton.com

F-Actin Probes in Living Cells

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Continued from Page 1

Continued References


Figures and Table on Page 3
**Figures and Table Appendix**

**Figures and Table Referenced in Text**

Table 1. Actin-binding probes for live cell imaging.

<table>
<thead>
<tr>
<th>Source</th>
<th>SiR/SPY-actins</th>
<th>Lifeact GFP tagged</th>
<th>Actin fluorescently labeled</th>
<th>Actin GFP tagged</th>
<th>Utophin GFP tagged</th>
<th>F-Tractin GFP tagged</th>
<th>Nanobody GFP tagged</th>
<th>Affimers GFP tagged</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Applications</strong></td>
<td>Live cell imaging of endogenous F-actin with WFI Ref. 14-18</td>
<td>SiR or SPY probes with desbromodesmethyl-jaspilakonolide</td>
<td>Skeletal muscle (rabbit) or non-muscle (beta actin human platelet)</td>
<td>Beta-actin fusion protein with GFP</td>
<td>Homo sapiens</td>
<td>Rattus norvegicus</td>
<td>Vicugna pacos</td>
<td>Synthetic act-in-binding probes isolated from phage library screens</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Direct application to cells and tissues Fluorogenic Cell Permeability (No transfection required) Photostability</td>
<td>Multiple Colors (e.g., far-red, red, orange, yellow, green)</td>
<td>Very similar conformation to endogenous actin Small fluorophore size.</td>
<td>Labeled actin is incorporated into endogenous filaments</td>
<td>Does not bind actin monomers (G-actin)</td>
<td>Does not bind actin monomers (G-actin)</td>
<td>Small probe size Low probability of affecting actin dynamics</td>
<td>High nanomolar affinity for F-actin</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Possible effects on actin dynamics at high concentrations</td>
<td>Large fluorescent reporter GFP Binds G-actin to produce a high background signal Possible effects on actin dynamics Requires injection</td>
<td>Requires injection</td>
<td>Large fluorescent reporter GFP Exogenous actin expression Binds G-actin to produce a high background signal Requires transfection</td>
<td>Large size Large fluorescent reporter GFP Possible effects on actin dynamics Requires transfection</td>
<td>Large fluorescent reporter GFP Possible effects on actin dynamics Requires transfection</td>
<td>Large fluorescent reporter GFP Binds G-actin to produce a high background signal Requires transfection</td>
<td>Large fluorescent reporter GFP Requires transfection</td>
</tr>
</tbody>
</table>

| Overall rating          | ++       | +++      | +++      | ++       | +++      | +++      | +++      | +++      |

Figure 1. MCF10A cells expressing H2B-GFP (blue) in Matrigel (3D culture) stained with SiR-actin (red). Image taken on an inverted LSM microscope. Courtesy of Christian Conrad and Katharina Jechow, Heidelberg.

Figure 2. SPY505 and SPY555 staining DNA, Actin, Tubulin, and DNA. Photo comes from front page of Spirochrome’s website.

Figure 3. SiR derivatives exist in equilibrium between the fluorescent zwitter-ionic (open) form (left structure) and the non-fluorescent spiro (closed) form (right structure).
**Cytoskeleton's Live Cell Imaging Tools**

### Live Cell Imaging Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Ex / Em</th>
<th>Amount</th>
<th>Cat. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPY555-Actin</td>
<td>555 / 580 nm</td>
<td>100 stains</td>
<td>CY-SC202</td>
</tr>
<tr>
<td>SPY505-DNA</td>
<td>512 / 531 nm</td>
<td>100 stains</td>
<td>CY-SC101</td>
</tr>
<tr>
<td>SPY555-DNA</td>
<td>555 / 580 nm</td>
<td>100 stains</td>
<td>CY-SC201</td>
</tr>
<tr>
<td>SPY555-Tubulin</td>
<td>555 / 580 nm</td>
<td>100 stains</td>
<td>CY-SC203</td>
</tr>
<tr>
<td>SPY595-DNA</td>
<td>599 / 615 nm</td>
<td>100 stains</td>
<td>CY-SC301</td>
</tr>
<tr>
<td>SPY650-DNA</td>
<td>652 / 674 nm</td>
<td>100 stains</td>
<td>CY-SC501</td>
</tr>
<tr>
<td>SPY650-Tubulin</td>
<td>652 / 674 nm</td>
<td>100 stains</td>
<td>CY-SC503</td>
</tr>
<tr>
<td>SPY700-DNA</td>
<td>696 / 718 nm</td>
<td>100 stains</td>
<td>CY-SC601</td>
</tr>
<tr>
<td>Sir-Actin™ Kit</td>
<td>630 / 680 nm</td>
<td>50 nmol</td>
<td>CY-SC001</td>
</tr>
<tr>
<td>Sir-Tubulin™ Kit</td>
<td>630 / 680 nm</td>
<td>50 nmol</td>
<td>CY-SC002</td>
</tr>
<tr>
<td>Cytoskeleton Kit</td>
<td>630 / 680 nm</td>
<td>50 nmol each</td>
<td>CY-SC006</td>
</tr>
<tr>
<td>Sir-DNA™ Kit</td>
<td>630 / 680 nm</td>
<td>50 nmol</td>
<td>CY-SC007</td>
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<tr>
<td>Sir700-Actin Kit</td>
<td>690 / 720 nm</td>
<td>35 nmol</td>
<td>CY-SC013</td>
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<tr>
<td>Sir700-Tubulin Kit</td>
<td>690 / 720 nm</td>
<td>35 nmol</td>
<td>CY-SC014</td>
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<tr>
<td>Sir700-DNA Kit</td>
<td>690 / 720 nm</td>
<td>35 nmol</td>
<td>CY-SC015</td>
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<tr>
<td>Flipper-TR™ Kit</td>
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<td>CY-SC020</td>
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**Exploring Cytoskeleton's Novel Kits**

### Acti-Stain™ Phalloidins

<table>
<thead>
<tr>
<th>Product</th>
<th>Amount</th>
<th>Cat. #</th>
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<tbody>
<tr>
<td>Acti-stain 488™ phallloid</td>
<td>300 Slides</td>
<td>PHDG1-A</td>
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<tr>
<td>Acti-stain 555™ phallloid</td>
<td>300 Slides</td>
<td>PHDH1-A</td>
</tr>
<tr>
<td>Acti-stain 670™ phallloid</td>
<td>300 Slides</td>
<td>PHDN1-A</td>
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<tr>
<td>Rhodamine Phalloid</td>
<td>1 x 500 µl</td>
<td>PHDR1</td>
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</table>

### Labeled Actin Proteins

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<tbody>
<tr>
<td>Rhodamine Actin Protein</td>
<td>4 x 10 µg</td>
<td>APHR-A</td>
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<tr>
<td></td>
<td>20 x 10 µg</td>
<td>APHR-C</td>
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<tr>
<td>Rhodamine Actin Protein</td>
<td>10 x 20 µg</td>
<td>AR05-B</td>
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<tr>
<td></td>
<td>20 x 20 µg</td>
<td>AR05-C</td>
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### Actin Biochem Kits

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<tr>
<td>Actin Binding Protein Spin-Down Assay Biochem Kit</td>
<td>30-100</td>
<td>BK001</td>
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<tr>
<td>Actin Polymerization Biochem Kit (fluorescence format)</td>
<td>30-100</td>
<td>BK003</td>
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<tr>
<td>Actin Binding Protein Spin-Down Assay Biochem Kit</td>
<td>30-100</td>
<td>BK013</td>
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<tr>
<td>G-Actin/F-actin In Vivo Assay Biochem Kit</td>
<td>30-100</td>
<td>BK037</td>
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<tr>
<td>Measure the distribution of monomer and polymer actin</td>
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</table>

### G-LISA Activation Assay Kits

<table>
<thead>
<tr>
<th>Product</th>
<th>Assays</th>
<th>Cat. #</th>
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<tbody>
<tr>
<td>RhoA G-LISA™ Activation Assay (Luminescence format)</td>
<td>96</td>
<td>BK121</td>
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<tr>
<td>RhoA G-LISA™ Activation Assay Kit (Colorimetric format)</td>
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<td>BK124</td>
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<tr>
<td>Rac1,2,3 G-LISA™ Activation Assay (Colorimetric format)</td>
<td>96</td>
<td>BK125</td>
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<tr>
<td>Rac1 G-LISA™ Activation Assay (Luminescence format)</td>
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<td>BK126</td>
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<tr>
<td>Rac1 G-LISA™ Activation Assay Kit (Colorimetric Based)</td>
<td>96</td>
<td>BK128</td>
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<tr>
<td>Ras G-LISA™ Activation Assay Kit (Colorimetric Based)</td>
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<td>BK131</td>
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<td>Total RhoA ELISA</td>
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### Pull Down Activation Assay Kits

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<tr>
<th>Product</th>
<th>Assays</th>
<th>Cat. #</th>
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<tr>
<td>Ras Pull-down Activation Assay Biochem Kit</td>
<td>50</td>
<td>BK008</td>
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<tr>
<td>RhoA / Rac1 / Cdc42 Activation Combo Biochem Kit</td>
<td>3 x 10</td>
<td>BK030</td>
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<td>Cdc42 Pull-down Activation Assay Biochem Kit</td>
<td>50</td>
<td>BK034</td>
</tr>
<tr>
<td>RhoA Pull-down Activation Assay Biochem Kit</td>
<td>80</td>
<td>BK036</td>
</tr>
</tbody>
</table>

**Featured Papers and Application Notes**


**Application Notes**

- "A Bright Dye for Live-Cell STED Microscopy"; S. Pitsch, I. Köster.