1. Introduction

■ About HMSiR-Halo

HMSiR-Halo is a fluorescent imaging probe for super-resolution imaging. This probe blinks spontaneously under the physiological conditions, without thiols, oxygen scavengers or irradiation of high-power laser, all of which were necessary for causing the blinking of fluorescent dyes in the previous dSTORM observation. Irradiation of 405 nm laser, which is needed for the observation with Alexa Fluor® 647, is not necessary with this probe. HMSiR-Halo has a chloroalkane base that specifically attaches to HaloTag® fusion proteins. HaloTag® fusion proteins in live cells are easily labeled by the probe.

2. Staining protocol of live cells with HMSiR-Halo

■ Procedure for labeling reaction

1. Dissolve HMSiR-Halo in DMSO to get 10 mM stock solution.
2. On the day before the observation, prepare cells expressing HaloTag® fusion proteins.
3. Add 100 nM HMSiR-Halo to the cultured cells, and incubate over night at the cultivate condition.
4. Wash cells with the culture medium, and replace the cells on the glass bottom dish.
5. Observe the cells with STORM about 3 hours after the replacement.

■ Fluorescent observation

Intensity of 647 nm laser for the evanescent field is 100 W/cm² by N-STORM (Nikon). 692/40 nm band pass emission filter (Semrock) is usable for the observation. Irradiation of 405 nm laser is not necessary. Observe cell in PBS without any additives. Capture 1000~5000 pictured as followed by the manual of the STORM.

■ Storage

Probes are packed under N₂ atmosphere, and are shipped at refrigerate state. After receipting, store under -20°C, desiccate and protect from light. We recommend using up DMSO solution of the dye.

Table 1. Product information

<table>
<thead>
<tr>
<th>Cot No.</th>
<th>Material</th>
<th>Amount</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A201-01</td>
<td>HMSiR-Halo</td>
<td>15 nmol</td>
<td>Store under -20°C, desiccate and protect from light. Use up its DMSO solution.</td>
<td>1 year (unopened)</td>
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<tr>
<td>A201-02</td>
<td></td>
<td>30 nmol</td>
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