

# Cellular Senescence Detection Kit - SPiDER-βGal

## Tips for a Successful Assay

### Troubleshooting



Problem	Causes and Solution
Background in control cells	<p>Reagents were not prepared correctly.</p> <p>Check that the reagents were stored correctly. SPiDER-βGal DMSO <b>stock solution</b> and Bafilomycin A1 DMSO <b>stock solution</b> is stable for 1 month at -20 °C. SPiDER-βGal <b>working solution</b> and Bafilomycin A1 <b>working solution can't be stored</b>. Be sure to use the working solution immediately.</p>
Background in senescent cells without adding SPiDER-βGal working solution	<p>Lipofuscin, which consists of autofluorescent granules, accumulated in the senescent cells.</p> <p>In order to achieve accurate SA-β-gal activity assay in senescent cells, we recommend to prepare samples without SPiDER-βGal staining. Please compare fluorescence intensity of both cells with / without SPiDER-βGal staining. For Microscopy or Flow Cytometry <b>Please visit product page and check "FAQ"</b>.</p>
Background in fix cells assay	<p>The incubation with SPiDER-βGal working solution was done in a 5% CO<sub>2</sub> incubator.</p> <p>We recommend not to use a 5% CO<sub>2</sub> incubator <b>during incubation with SPiDER-βGal working solution</b>. If incubation is done in a 5% CO<sub>2</sub> incubator, the pH of the buffer may become acidic. Acidic pH results in higher background from the endogenous β-galactosidase activity and it would be difficult to distinguish between control cells and senescent cells. <b>Please incubate the plate in a dry incubator without CO<sub>2</sub></b>.</p>
No difference in fluorescence intensity between senescent cells and control cells.	<p>Cellular senescence wasn't induced.</p> <p>Please prepare for positive control. <b>Please visit product page and check "Positive Control"</b>.</p>
Low fluorescence reading	<p>Incubation time with SPiDER-βGal working solution was short.</p> <p><b>Optimize the incubation time (~ 60 min).</b></p> <p>Concentration of SPiDER-βGal working solution was low.</p> <p><b>Optimize the concentration (~2 times the concentration described in the technical manual).</b></p>

For questions not addressed here, please contact us.  
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