

DATASHEET

F-Actin BufferKit

F-Actin BufferKit

Modulation of actin dynamics

Quantity: 1x
Cat. #: 5300-01

For Use in Research Only.
Not for Use in Diagnostic Processes.

Product Description

The BufferKit was developed for microplate assays and is applicable whenever actin dynamics should be modulated. The kit consists of individual components generally used to polymerize actin: the F-Actin Buffer contains Imidazole pH 7.4 to polymerize actin at physiological pH, and ATP required for the polymerisation process. The potassium chloride solution is used to polymerize actin and a magnesium chloride solution to promote nucleation.

While the F-Actin Buffer is added to each sample as a 10x stock, KCl and MgCl₂ can be added as single components or in combination. A final concentration of 100mM KCl and 2mM MgCl₂ is frequently used to polymerize actin and available as PolyMix, a premixed 10x stock solution with (Cat no.: 5000-01).

The F-Actin BufferKit allows measuring actin dynamics in the presence of e.g. 20-100mM KCl or in the absence of KCl and the presence of e.g. 2mM MgCl₂ only. A combination of both salts to a final concentration of 100mM KCl and 2mM MgCl₂, results in fast nucleation and polymerisation at e.g. 9.5µM actin. Hence, the actin polymerization is significantly slower at concentrations of e.g. 50-100mM KCl only. Thus, in the presence of e.g. 70mM KCl the addition of actin nucleators significantly affects the lag- and elongation-phase investigated by microplate fluorimetry.

Product Handling

The F-Actin BufferKit is used to polymerize 40ml of actin and is sufficient for two 96-well microplate assays.

- F-Actin Buffer (4x1.0ml lyophilized, liquid made with H₂O Milli-Q™, 0.22µm filtered solution)

Add 0.5ml of H₂O to the tube with F-Actin Buffer and vortex until dissolved. Transfer to a new tube and add another 0.5ml of H₂O and vortex. The buffer is now ready-to-use as a 10x stock solution.

- 1M KCl (1x 8.0ml, liquid in H₂O Milli-Q™, 0.22µm filtered solution)

Use at a final concentration of e.g. 20-100mM KCl.

For product inquiries please contact:

cusserv@hypermol.com
Fon: +49 (0)521 9876228
Fax: +49 (0)521 9876231
www.hypermol.com

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- 100mM MgCl₂ (1x5.0ml liquid in H₂O Milli-Q™, 0.22µm filtered solution)

Use at a final concentration of e.g. 1-2mM MgCl₂.

Example for sample pipetting 200µl/well

Add 80µl Pyrene-Actin (10%, 1.0mg/ml) to make a 9.52µM solution

+ 76µl H₂O

+ 20µl 10x F-Actin Buffer – mix well

The following components are add only before reading is started!

+ 20µl KCl (1M) to obtain a final concentration of 100mM

+ 4µl MgCl₂ (100mM) to obtain a final concentration of 2mM;

The conditions to polymerize actin have to be determined individually. To determine the most suitable condition for your assay, it is recommendable to measure polymerization with different KCl concentrations with or without MgCl₂ and to adjust the parameters of the device.

Best use a multichannel pipette and premix Pyrene-Actin, H₂O and F-Actin Buffer to fill a the V-shaped reservoir for easier loading. KCl and MgCl₂ are supplied in a larger volume than needed for reproducible loading.

Buffer Composition

1.0ml of F-Actin Buffer (10x) contains: 100mM Imidazole-Cl pH 7.4, 10mM ATP.

Product Storage and Stability

Store the lyophilized F-Actin Buffers at –70°C upon arrival. The liquids are stored at RT and are stable for at least two years. F-Actin Buffer will be stable in performance for at least 6 months from the date of purchase. Reconstituted F-Actin Buffer is kept on ice and should be used within 5 days.

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