Technical note No. 3812 Rev. 1.0

Vitabright stains – for cytometric profiling of apoptosis and other pathological processes

Table 1. Contents and storage

<table>
<thead>
<tr>
<th>Material</th>
<th>Size</th>
<th>Concentration</th>
<th>Storage</th>
<th>Stability</th>
<th>Ex/Em</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitabright-43™ (910-3019)</td>
<td>100 µl</td>
<td>1.0 mM ~ 274 µg/ml (100x)</td>
<td>&lt; 5°C, protect from light and moisture</td>
<td>1 month</td>
<td>361/431 nm</td>
</tr>
<tr>
<td>Vitabright-48™ (910-3020)</td>
<td>100 µl</td>
<td>1.0 mM ~ 298 µg/ml (100x)</td>
<td>light and moisture</td>
<td>1 month</td>
<td>383/485 nm</td>
</tr>
</tbody>
</table>

*VitaBright stains are dissolved in DMSO and each vial provides sufficient material for approximately 1000 assays, based on the following protocol.

*When stored as directed unopened vials are stable for at least 1 year. However, opened vials should be discarded if not used within 1 month.

*Approximate fluorescence excitation/emission maxima. Note that both UV and violet lasers can be used for excitation of VitaBright stains.

Introduction

Vitabright-43™ and -48™ are cell-permeable maleimide derivatives that react with thiol groups on proteins to give thioester-coupled fluorescent products (Figure 1)\(^1,2\). Free thiols play a number of important roles in biology. An example is the predominant cellular oxidant, reduced glutathione (GSH), which protects against oxidative damage. GSH provides a large proportion of the reducing power available in the cell, and its oxidation status largely determines the thiol-disulfide status of the cell and, hence, the cellular redox potential\(^3\). The concentration of GSH has been found to decrease upon induction of apoptosis, also when using non-oxidative apoptogenic agents, due to extrusion of GSH\(^4-7\).

![Figure 1: A) Schematic drawing showing the principle of intracellular detection of thiols using VitaBright Stains. Upon addition to a cell culture VitaBright crosses the cell membrane and immediately reacts with intracellular thiols, forming a blue fluorescent compound. B) CHO cells stained with VitaBright-48™ (blue) and a nuclear marker (red).](image)

Interchange reactions links the cellular concentration of GSH directly to the level of other reduced thiols. Moreover, the level of GSH, and hence the level of thiols, decreases in response to induction of apoptosis, thus measuring the level of free thiols may be used to quantify apoptosis. The two thiol probes VitaBright-43™...
and -48™ provide a very rapid, easy and reliable way of assaying apoptosis by either flow or image cytometry as no incubation or washing steps are required; this facilitates preservation of fragile apoptotic cells often lost during washing steps. To discriminate between necrotic and apoptotic cells VitaBright staining should be combined with an impermeable stain, such as propidium iodide. Using multicolor cytometry it has been demonstrated that VitaBright-43™ staining correlates well with phosphatidylserine externalization and Caspase 3/7 activity (figure 2)².

*Figure 2:* Multiplex assays demonstrating that VitaBright-43™ staining correlates well with phosphatidylserine externalization and Caspase 3/7 activity; as apoptosis progresses Annexin V CF-647 and NucView 488 signals increase while VitaBright-43™ signal decreases. A) Jurkat cells treated with 5 μM camptothecin (CPT) for 0, 2 and 4 hours, respectively. Cells were stained with VitaBright-43™, Annexin V CF-647 and SYTOX green and analysed by image cytometry using the NucleoCounter NC-3000 system. Plots show VitaBright-43™ intensity versus Annexin V CF-647 intensity. Nonviable cells were gated out based on SYTOX green uptake. Right panel shows an image of the CPT-treated sample. B) WeHi-S cells treated with 10 ng/μl TNF-α for 0, 2 and 4 hours, respectively. Cells were stained with VitaBright-43™, NucView 488 and propidium iodide and analysed by image cytometry. Plots show VitaBright-43™ intensity versus NucView 488 intensity. Nonviable cells were gated out based on propidium iodide uptake. Right panel shows an image of the TNF-α treated sample.

VitaBright-48™ may also be used for measuring apoptosis. However, this probe detects changes in thiol level, and hence apoptosis, later than VitaBright-43™. Table 2 summarizes some of the features of the two thiol probes.

**Table 2. Comparison of VitaBright-43™ and -48™**

<table>
<thead>
<tr>
<th>Thiol Probe</th>
<th>Signal strength</th>
<th>Apoptosis</th>
<th>Staining kinetics</th>
<th>Organisms tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitabright-43™</td>
<td>Bright</td>
<td>Early to mid-stage</td>
<td>Fast, no incubation</td>
<td>Mammalian and insect cells</td>
</tr>
<tr>
<td>Vitabright-48™</td>
<td>Very bright</td>
<td>Mid-stage to late</td>
<td>Fast, no incubation</td>
<td>Mammalian, insect and yeast cells</td>
</tr>
</tbody>
</table>
Since no single parameter defines apoptosis it is recommended to use a combination of different markers for reliable detection of apoptosis (refer to www.chemometec.com for alternative product for apoptosis research).

**Experimental Protocol**

The following protocol was developed with Jurkat, WeHi, U2OS and MCF-7 cells with an optimized concentration of VitaBright-43™ or -48™ of 10 µM, but it can be adapted for any mammalian cell type. Growth medium, cell density, cell type variants and other factors may influence labelling. It is recommended to test a concentration range of the particular VitaBright to estimate the optimal conditions for the used model.

**Materials required**

- Cells (appropriate sample concentrations range from $5 \times 10^5$ to $5 \times 10^6$ cells/ml)
- Complete medium for the cell type used
- VitaBright-43™ (910-3019) or VitaBright-48™ (910-3020)
- Cell-impermeable stain, e.g. propidium iodide (910-3016)
- Optional: phosphate buffered saline (PBS)

**Cell staining**

1. Induce apoptosis in cell by desired method. Include a control sample of untreated cells.

2. Harvest cells and aliquot 100 µl sample per tube.

3. Add VitaBright to tubes at a final concentration of 10 µM.
   - Note: the optimal dye concentration should be determined empirically.

4. Add cell impermeable dye to stain non-viable cells
   - Eg. add propidium iodide to a final concentration of 10 µg/ml.

5. Quantify cellular fluorescence by image or flow cytometry within 15 minutes

6. To identify apoptotic cells exclude non-viable cells and score cells with dim blue fluorescence.
References


Handling and storage
For handling and storage of ChemoMetec reagents refer to the corresponding product documentation. For other reagents refer to the material data sheet from the manufacturer of the reagents and chemicals.

Warnings and precautions
For safe handling and disposal of the ChemoMetec reagents refer to the corresponding product documentation. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

Limitations
The Chemometec reagents are FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

Liability disclaimer
This note is for RESEARCH PURPOSES ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

Product disclaimer
ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

Copyright
Copyright © ChemoMetec A/S 2010. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written consent of ChemoMetec A/S, Gydevang 43, DK-3450 Allerod, Denmark.