

### 2.1.3 Summary of methods for studying cell proliferation and cell viability in cell populations

#### DNA Synthesis

Method/Roche Applied Science product	Label	Assay Principle	Advantages	Limitations	For product information, see
<b>[<sup>3</sup>H]-TdR Proliferation Assay</b>	[ <sup>3</sup> H]-TdR	<ul style="list-style-type: none"> <li>• [<sup>3</sup>H]-TdR is added to cells cultured in MTP and the cells are incubated (usually for 2–24 h). During this labeling period, [<sup>3</sup>H]-TdR is incorporated into the DNA of proliferating cells.</li> <li>• Cells are harvested by vacuum aspiration onto glass fiber filters. While free [<sup>3</sup>H]-TdR is washed through the filters, the [<sup>3</sup>H]-TdR incorporated in the DNA is retained.</li> <li>• The radioactivity retained on the filters is measured by liquid scintillation counting (LSC).</li> </ul>	<ul style="list-style-type: none"> <li>• Sensitive (10<sup>3</sup>–10<sup>4</sup> cells/test required)</li> <li>• Linear measurement of cell proliferation over a broad, logarithmic range</li> <li>• Low background</li> </ul>	<ul style="list-style-type: none"> <li>• Radioactive isotope handling and storage problems</li> <li>• Long half life</li> <li>• Radioactive waste disposal costs</li> </ul>	
<b>BrdU incorporation assay</b> <b>BrdU Labeling and Detection Kit III</b>	BrdU	<ul style="list-style-type: none"> <li>• BrdU is added to cells cultured in MTP and the cells are incubated (usually for 2–24 h). During this labeling period BrdU is incorporated into the DNA of proliferating cells.</li> <li>• After the culture supernatant is removed, the cells are fixed and then incubated with an anti-BrdU antibody conjugated with peroxidase (anti-BrdU-POD).</li> <li>• This antibody binds to BrdU which has been incorporated into the DNA.</li> <li>• Bound anti-BrdU-POD is detected by a substrate reaction and quantified by an ELISA plate reader.</li> </ul>	<ul style="list-style-type: none"> <li>• No transfer of the cells; the entire assay is performed in a single MTP</li> <li>• Non-radioactive</li> </ul>	<ul style="list-style-type: none"> <li>• Assay is not linear over a broad logarithmic range of cell proliferation (limitation of the ELISA plate reader)</li> <li>• 3 washing and incubation steps</li> <li>• Longer assay time</li> </ul>	page 91-93 of this guide
<b>BrdU incorporation assay</b> <b>Cell Proliferation ELISA, BrdU (colorimetric)</b>	BrdU	See above (BrdU incorporation assay)	<ul style="list-style-type: none"> <li>• No transfer of the cells; the entire assay is performed in a single MTP</li> <li>• 1 washing and 2 incubation steps only</li> <li>• Short assay time</li> <li>• Robust system: low standard deviation</li> <li>• Sensitive (10<sup>3</sup>–10<sup>4</sup> cells/test required)</li> </ul>	<ul style="list-style-type: none"> <li>• Assay is not linear over a broad logarithmic range of cell proliferation (limitation of the ELISA plate reader)</li> </ul>	page 94-97 of this guide
<b>BrdU incorporation assay</b> <b>Cell Proliferation ELISA, BrdU (chemiluminescence)</b>	BrdU	See above (BrdU incorporation assay)	<ul style="list-style-type: none"> <li>• [see also Cell Proliferation ELISA, BrdU (colorimetric)]</li> <li>• Linear measurement of cell proliferation over a broad, logarithmic range</li> </ul>	<ul style="list-style-type: none"> <li>• For chemiluminescence measurement special MTP (Black with clear, flat bottom) required</li> </ul>	page 94-97 of this guide

▲ Table 15: Summary of methods to study DNA synthesis in cell populations.

#### Metabolic activity

Method/Roche Applied Science product	Label	Assay Principle	Advantages	Limitations	For product information, see
<b>MTT Assay<sup>61</sup></b> <b>Cell Proliferation Kit I (MTT)</b>	Non-isotopic	<ul style="list-style-type: none"> <li>• MTT solution is added to cells cultured in MTP and the cells are incubated (usually for 4 h). During this period, MTT is converted into a colored, water-insoluble formazan salt by the metabolic activity of viable cells.</li> <li>• The insoluble formazan is solubilized.</li> <li>• The amount of formazan is quantified by an ELISA plate reader at 550–600 nm.</li> </ul>	<ul style="list-style-type: none"> <li>• No transfer of the cells; the entire assay is performed in a single MTP</li> <li>• MTT is metabolized by all cells; the assay can be used with all cell types</li> <li>• Inexpensive</li> </ul>	<ul style="list-style-type: none"> <li>• Assay is not linear over a broad logarithmic range of cell proliferation due to the ELISA plate reader</li> <li>• Insoluble reaction product: resolubilization of the reaction product required</li> <li>• Cannot take multiple time points in a single assay</li> <li>• Cells with low metabolic activity (e.g., lymphocytes) must be used in high numbers</li> </ul>	page 82-88 of this guide
<b>XTT Assay<sup>62</sup></b> <b>Cell Proliferation Kit II (XTT)</b>	Non-isotopic	<ul style="list-style-type: none"> <li>• XTT solution is added to cells cultured in MTP and the cells are incubated (usually for 2–4 h). During this period, XTT is converted into a colored, soluble formazan salt by the metabolic activity of viable cells.</li> <li>• The amount of formazan is quantified by an ELISA plate reader at 450–500 nm.</li> </ul>	<ul style="list-style-type: none"> <li>• No transfer of the cells; the entire assay is performed in a single MTP</li> <li>• Soluble reaction product</li> <li>• Can take multiple time points in a single assay</li> </ul>	<ul style="list-style-type: none"> <li>• Assay is not linear over a broad logarithmic range of cell proliferation due to the ELISA plate reader</li> <li>• XTT working solution has to be prepared shortly before use</li> <li>• XTT is not metabolized by all cell types</li> </ul>	page 82-88 of this guide
<b>WST-1 Assay</b> <b>Cell Proliferation Reagent (WST-1)</b>	Non-isotopic	<ul style="list-style-type: none"> <li>• WST-1 solution is added to cells cultured in MTP and the cells are incubated (usually for 0.5–2 h). During this period, WST-1 is converted into a colored, soluble formazan salt by the metabolic activity of viable cells.</li> <li>• The amount of formazan is quantified by an ELISA plate reader at 420–480 nm.</li> </ul>	<ul style="list-style-type: none"> <li>• No transfer of the cells; the entire assay is performed in a single MTP</li> <li>• Soluble reaction product</li> <li>• Repeated measurement of the assay</li> <li>• Ready-to-use solution</li> </ul>	<ul style="list-style-type: none"> <li>• Assay is not linear over a broad logarithmic range of cell proliferation due to the ELISA plate reader</li> <li>• WST-1 is not metabolized by all cell types</li> </ul>	page 82-88 of this guide

▲ Table 16: Summary of methods to study metabolic activity in cell populations.

### 2.1.4 Single reagents for the measurement of DNA synthesis

Product	Cat. No.	Pack Size
<b>FixDenat</b>	11 758 764 001	4 x 100 ml (2000 tests)
<b>Anti-Bromodeoxyuridine-Peroxidase, Fab fragments, formalin grade</b>	11 585 860 001	15 U**

◀ Table 17: Single reagents available for detection of DNA fragmentation.

\* Flow cytometry  
\*\* 0.2 U/ml (ELISA), 1.5 U/ml (Immunocytochemistry)

Product	Cat. No.	Pack Size
<b>Anti-Bromodeoxyuridine formalin grade</b>	11 170 376 001	50 µg (250 tests*)
<b>Anti-Bromodeoxyuridine-Fluorescein formalin grade</b>	11 202 693 001	50 µg (100 tests**)

