

### 1.3 Technical tips on the use of Annexin-V-Biotin for light microscope detection

The following protocol provides a method for the detection of Annexin-V-Biotin-binding to cell culture cells with light microscopy. The percentage of necrotic cells is determined by trypan blue staining.

#### Preparation of solutions

- Annexin-V-Biotin working solution: Dilute 20  $\mu\text{l}$ . Annexin-V-Biotin labeling reagent in 1000  $\mu\text{l}$  incubation buffer (sufficient for 10 samples).
- HEPES buffer: Prepare according to the instructions in the Annexin-V-Biotin pack insert.

All steps can be performed at room temperature

- 1 Incubate  $1 \times 10^6$  cells in 100  $\mu\text{l}$  Annexin-V-Biotin working solution for 10–15 min.
- 2 Wash 2 times with HEPES buffer.  
For suspension cells: Continue with step 3.  
For adherent cells: Continue with step 4.
- 3 Resuspend suspension cells in 1 ml HEPES buffer. Transfer  $2 \times 10^5$  cells to a slide using cytopsin device.
- 4 Air dry cells. Fix with methanol/ethanol 1:1 for 90 sec.
- 5 Air dry cells. Add 100  $\mu\text{l}$  Streptavidin-POD (Cat. No. 11 089 153 001) working solution, incubate for 1 h.
- 6 Rinse with HEPES buffer
- 7 Add DAB substrate solution (Cat. No. 11 718 096 001) working solution, incubate for 10–15 min.
- 8 Rinse with HEPES buffer
- 9 Analyze samples under a light microscope.

