

# CASPASE-3 ASSAY

Harvest cells

Cells that have become detached should be collected.

Discarding detached cells will cause an underestimation of the amount of apoptosis.

Transfer the medium to a 15-ml conical centrifuge tube, wash w/ PBS, add trypsin.

Transfer cells to the tube and centrifuge 5 min at 800 x g, r.t.

Remove supernatant and wash the pellet w/ PBS.

Centrifuge 5 min at 800 x g, r.t.

Remove supernatant and resuspend the pellet in ice-cold lysis buffer (100 ul or in a volume equal to that of the pellet)

Incubate sample 20 min on ice, then lyse cells (3 cycles of freeze-thaw/dry-ice-37C).

Transfer lysates to a microcentrifuge tube and centrifuge 3 min at 16000 x g 4C, to remove the nuclei..

Collect supernatant (cytosol) and use an aliquot to determine its protein concentration (bicinchoninic acid method 1ul is enough).

Dilute all samples to 2µg/µl if possible.

Freeze the cytosol in 50 µl aliquots at -70C.

Caspase-3 assay w/ 100 µg proteins (dilute to 50 µl).

Add 50 µl of ASSAY BUFFER (2x w/DTT 100x)

Add 5µl substrate

Incubate for 1h 30 min at 37C.

Reader EX 420 EM 530 Gain 60

## **LYSIS BUFFER 500 ml**

<b>10 mM TRIS, pH 7.3</b>	50 ml 0.1 M TRIS, pH 7.3 (1)
<b>10 mM NaH<sub>2</sub>PO<sub>4</sub></b>	10 ml 0.5 M NaH <sub>2</sub> PO <sub>4</sub>

<b>150 mM NaCl</b>	15 ml 5M NaCl
<b>1% Triton X-100</b>	50 ml Triton X-100 10%
	Add water to 400 ml pH 5.8 Adjust pH to 6.6 with NaOH 0.1N at 4°C Add water to 500 ml

### **ASSAY BUFFER 2X 500 ml**

<b>20 mM HEPES, pH 7.4</b>	20 ml 0.5M HEPES, pH 7.4 <sup>(2)</sup>
<b>100 mM NaCl</b>	10 ml 5M NaCl
<b>1 mM EDTA</b>	1 ml 0.5 M EDTA, pH 8
<b>0.2% CHAPS</b>	1 g CHAPS
<b>20% glycerol</b>	100 ml Glycerol
	Add water to 400 ml pH 7.2 r.t. Adjust pH to 7.4 with NaOH 0.1N Add water to 500 ml

(1) Tris 1M: 12.14g Trizma (Sigma T1503) I 80 ml water  
Adjust pH with HCl conc ( $\pm$  8 ml)  
Mix and add water to 100 ml

(2) HEPES 0.5M: 5.95 gr in 50m ml water  
Adjust pH

### **1 mM AC-DEVD-AFC in DMSO (BIOSOURCE)**

5 mg dissolve in 6.86 ml DMSO

### **20 mM Z-VAD-FMK in DMSO**

1 mg in 107.05  $\mu$ l DMSO