

# FasL-Strep

## Apoptosis assay protocols

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# 1 Homogenous assay protocol

## 1.1 Human FasL-Strep

1. Grow a Jurkat A3 permanent human T-cell line (e.g. cat. no. CRL2570, ATCC) in flasks with RPMI 1640-medium + GlutaMAX (GibCo) supplemented with 10 % FBS (Biochrom), 100 units/ml Penicillin and 100  $\mu\text{g/ml}$  Streptomycin (GibCo). Jurkat A3 grow in suspension.
2. Seed 100,000 cells per well (100  $\mu\text{l}$ ) into a 96-well microtiterplate (e.g. cell culture microtiterplate, TPP, cat. no. 92696).
3. Add 100  $\mu\text{l}$  human FasL-Strep to the wells (e.g. at a constant concentration of 250 ng/ml; final concentration in the well: 125 ng/ml).

If the extent of apoptosis induced by FasL-Strep should be modified, a titration of FasL-Strep employing 2-fold dilution steps starting from 1  $\mu\text{g/ml}$  (final concentration) can be tested.

4. Incubate for 3h at 37°C.
5. Lyse cells by adding 20  $\mu\text{l}$  lysis buffer (250 mM HEPES, 50 mM  $\text{MgCl}_2$ , 10 mM EGTA, 5 % Triton-X-100, 100 mM DTT, 10 mM AEBSF, pH 7.5) per well. Mix thoroughly the cell suspension by pipetting up and down, and incubate on ice for at least 30 minutes.
6. Determine the extent of apoptosis by cleavage of the specific caspase 3/7 substrate Ac-DEVD-AFC (Biomol) as apoptosis is paralleled by an increased activity of effector caspases (e.g. caspase 3). For this purpose, transfer 20  $\mu\text{l}$  of the cell lysate to a black 96-well microtiterplate. After the addition of 80  $\mu\text{l}$  buffer (50 mM HEPES, 1 % Sucrose, 0.1 % CHAPS, 50  $\mu\text{M}$  Ac-DEVD-AFC, and 25 mM DTT, pH 7.5) transfer the plate to a microtiterplate reader and monitor the increase in fluorescence intensity (excitation wavelength 400 nm, emission wavelength 505 nm).

Optional: To increase the apoptotic effect of FasL-Strep, the antibody StrepMAB-Imm0 may be added additionally in step 3 at a concentration of 10  $\mu\text{g/ml}$  to crosslink FasL-Strep.

## 1.2 Murine FasL-Strep

The assay can be carried out in analogy to the assay with human FasL-Strep (see Chapter 1.1) as the human Jurkat A3 cell line is also sensitive to murine FasL-Strep.

Alternatively, the adherent P815 mouse cell line can be taken for the assay with murine FasL-Strep:

1. Grow the permanent mouse mastocytoma cell line P815 (cat. no. TIB-64, ATCC) in flasks with DMEM supplemented with 10 % FBS (Biochrom), 1 mM Na-Pyruvate, 100 units/ml Penicillin and 100  $\mu\text{g/ml}$  Streptomycin (GibCo).
2. Seed 25,000 cells per well (100  $\mu\text{l}$ ) into a 96-well microtiterplate (e.g. cell culture microtiterplate, TPP, cat. no. 92696) on the day before the apoptosis assay to allow cells to adhere.
3. Add 100  $\mu\text{l}$  mouse FasL-Strep to the wells (e.g. at a constant concentration of 250 ng/ml; final concentration in the well: 125 ng/ml)

If the extent of apoptosis induced by FasL-Strep should be modified, a titration of FasL-Strep employing 2-fold dilution steps starting from 1  $\mu\text{g/ml}$  (final concentration) can be tested.

4. Incubate for 6 h at 37°C.
5. Lyse cells by adding 20  $\mu\text{l}$  lysis buffer (250 mM HEPES, 50 mM  $\text{MgCl}_2$ , 10 mM EGTA, 5 % Triton-X-100, 100 mM DTT, 10 mM AEBSF, pH 7.5) per well, thoroughly mix by pipetting up and down, and incubate on ice for at least 30 minutes.
6. Determine the extent of apoptosis by cleavage of the specific caspase 3/7 substrate Ac-DEVD-AFC (Biomol) as apoptosis is paralleled by an increased activity of effector caspases (e.g. caspase 3). For this purpose, transfer 20  $\mu\text{l}$  of the cell lysate to a black 96-well microtiterplate. After the addition of 80  $\mu\text{l}$  buffer (50 mM HEPES, 1 % Sucrose, 0.1 % CHAPS, 50  $\mu\text{M}$  Ac-DEVD-AFC, and 25 mM DTT, pH 7.5) transfer the plate to a microtiterplate reader and monitor the increase in fluorescence intensity (excitation wavelength 400 nm, emission wavelength 505 nm).

Optional: To increase the apoptotic effect of FasL-Strep, the antibody StrepMAB-Immo may be added additionally in step 3 at a concentration of 10  $\mu\text{g/ml}$  to crosslink FasL-Strep.

## 2 Heterogenous assay

1. Grow a Jurkat A3 permanent human T-cell line (e.g. cat. no. CRL2570, ATCC) in flasks with RPMI 1640-medium + GlutaMAX (GibCo) supplemented with 10 % FBS (Biochrom), 100 units/ml Penicillin and 100 µg/ml Streptomycin (GibCo).
2. Transfer 100 µl of human FasL-Strep to the wells of a Strep-Tactin microtiterplate (cat. no. 2-1501-001, IBA) (e.g. at a constant concentration of 50 ng/ml; final concentration in the well: 25 ng/ml after addition of the cells following step 3); incubate for 30 min at 37°C.

If the extent of apoptosis induced by FasL-Strep should be modified, a titration of FasL-Strep employing 2-fold dilution steps starting from 200 ng/ml (final concentration) can be tested.

3. Add 100,000 cells per well (100 µl).
4. Incubate for 3h at 37°C.
5. Lyse cells by adding 20 µl lysis buffer (250 mM HEPES, 50 mM MgCl<sub>2</sub>, 10 mM EGTA, 5 % Triton-X-100, 100 mM DTT, 10 mM AEBSF, pH 7.5) per well, thoroughly mix the cell suspension by pipetting up and down, and incubate on ice for at least 30 minutes.
6. Determine the extent of apoptosis by cleavage of the specific caspase 3/7 substrate Ac-DEVD-AFC (Biomol) as apoptosis is paralleled by an increased activity of effector caspases (e.g. caspase 3). For this purpose, transfer 20 µl of the cell lysate to a black 96-well microtiterplate. After the addition of 80 µl buffer (50 mM HEPES, 1 % Sucrose, 0.1 % CHAPS, 50 µM Ac-DEVD-AFC, and 25 mM DTT, pH 7.5) transfer the plate to a microtiterplate reader and monitor the increase in fluorescence intensity (excitation wavelength 400 nm, emission wavelength 505 nm).

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