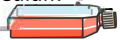
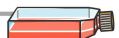
Preparation of samples

Growing the cells in full medium
Until 80–90 % of confluence



Growing the cells in serum-free medium for 48-72 h



Cell medium collection
at 4 °C

Cell debris removal
centrifugation 20 min, 7000 x *g*, 4 °C

STOP POINT 1 (store at -20 °C)

Medium concentration using ultracentrifugal Filter with 10 kDa molecular weight cutoff

STOP POINT 2 (store at -80 °C)

Protein concentration measurement Bradford or BCA method

STOP POINT 3 (store at -80 °C)

SDS-PAGE

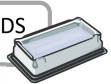
SDS-PAGE samples preparation
+ 2x non-reducing loading buffer,
20 min incubation at 37 °C

STOP POINT 4 (store at -80 °C)

Preparation of the gel with gelatin
+ at least 12 h incubation at 4 °C

Running electrophoresis
20 – 40 mA per gel at 4 °C

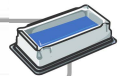
Gel washing – removing of SDS
2 x 30 min at RT in washing buffer



Gel activation
12 h incubation at 4 °C in incubation buffer

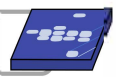
Gel staining and analysis

Staining of the gel
30 min at RT in Coomassie Brilliant Blue solution



Destaining of the gel
at RT in destaining buffer

Gel visualization with dedicated equipment



STOP POINT 5

Analysis of the gelatin digested area ImageJ (Fiji) software

Analysis of band intensity ImageLab software