

## In vitro Transcription/ Translation Protocol

### Sample mix

40 ul Master Mix

2ul s35

vary last 8 ul between DNA and water depending on amount of DNA needed

### luc mix

20 ul master mix

1 ul s35

2 ul DNA

2ul Water

- 1) Thaw out and mix DNA/ nuclease free water.  
(have a luc control reaction that is half the size of the sample reactions)
- 2) Thaw out Master mix quickly in your hand and then put on ice, add to DNA mix.
- 3) Add S35 to the mix and incubate at 30C for 2 hours.
- 4) While incubating pour an appropriate conc. SDS-Acrylamide gel.
- 5) For first time Transcript/Translate run 5 ul of product on gel, further times can run 2-3ul. Denature for 5 mins in 20 ul SDS loading buffer.
- 6) After running gel either immediately dry the gel or follow the Autofluor protocol outlined below.
- 7) Expose gel to IP plate over night.

## Autofluor/SDS-PAGE Gel Fixation Protocol

- 1) After electrophoresis, fix gel in 5% glacial acetic acid, 5% 2-propanol, 90% dH<sub>2</sub>O (approx. 20 ml) for 15-20 mins.
- 2) Decant and wash under continuously flowing tap water for 15 mins.
- 3) Cover with Autofluor/ tin foil and rock @ RT for 30 min / 1 of gel thickness.
- 4) Dry immediately for 30 mins at 80C.