Aβ detection, immunoblot

HEK293 APPswe - Huang et al., J Neurosci 2016

Detection of Aβ in conditioned media by immunoblot requires concentration (using a microcon system, or by TCA) – which requires conditioning in FBS-free media (FBS will interfere with protein concentration methods). As Aβ monomers are small (<10kDa), precipitates or concentrated media are resolved on 10-20% Tricine gradient gels. Other secreted or shed extracellular factors (sAPPα, sSORLA) are more abundant, and may not require concentration steps for detection by immunoblot.

Steps:
1) Wash cells twice with plain DMEM (no FBS) for 4-16h before harvest (incubate in 1ml DMEM ea. 6-well plate; typically media is conditioned overnight).
2) TCA precipitate proteins in the conditioned media; lyse cells and immunoblot lysates for proteins of interest.
3) Dissolve pellet with Tricine loading buffer.
4) Run 10-20% Tricine gel, use Tricine running buffer.
5) Transfer (immunoblot) onto nitrocellulose or PVDF.
6) Incubate the membrane in 0.2% Glutaradehyde for 30mins.
7) Block membranes with 5% milk for 1h.
8) Probe for Aβ using the 6E10 antibody.

TCA precipitation

Steps
1) Add 2% Na-deoxycholate to 0.02% (100x) to the FBS-free conditioned media
2) Mix at RT (room temperature) for 15 mins.
3) Add 100% TCA to a final concentration of 10%, incubate rocking 1h at RT.
4) Spin 4°C for 10 mins. (max speed), remove supernatant and dry.
5) Add 200µls of cold acetone to TCA pellet, incubate on ice 15 mins.
6) Spin 4°C for 10 mins at max speed.
7) Remove supernatant and dry tubes, resuspend in Tricine running buffer.

Note: for larger proteins, standard SDS-PAGE Tris/glycine gels can be used