

Materials List for:

Use of Two Dimensional Semi-denaturing Detergent Agarose Gel Electrophoresis to Confirm Size Heterogeneity of Amyloid or Amyloid-like Fibers

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Materials

Name	Company	Catalog Number	Comments
gel electrophoresis unit	Fisher	HE99XPRO	apparatus for gel running.
agrose	VWR	97062-250	For agarose gel.
paper tower	Fisher	19-120-2484	for transferring
filter paper	VWR	21427-386	for transferring
PVDF membrane	Bio-Rad	162-0177	for transferring
blocking milk powder	Bio-Rad	1706404XTU	for blocking in Western blot
MLKL antibody	Genetex	GTX107538	rabbit anti-MLKL antibody
RIPK1 antibody	BD Biosciences	610459	mouse anti-RIPK1 antibody
RIPK3 antibody	gift from Dr. Xiaodong Wang		rabbit anti-RIPK3. See reference 11.
anti-Rabbit-HRP	Sigma	A6154	secondary antibody
ECL	Fisher	WBKLS0500	Western chemiluminescent HRP substrate
X-ray film	Fisher	F-BX810	for Western blot result
DMEM	Sigma	D6429	for cell culture
fetal bovine serum	Sigma	F4135	for cell culture
penicilin-streptomycin	Sigma	P4333	for cell culture
Trypsin solution	Sigma	T4049	for cell culture
PBS for tissue culture	Sigma	D8662	for cell culture
recombinant TNF	made in our lab		for inducing necroptosis. See reference 11.
smac-mimetic	gift from Dr. Xiaodong Wang		for inducing necroptosis. See reference 11.
ZVAD-FMK	ApexBio	A1902	for inducing necroptosis. See reference 11.
Cell Counter	Bio-Rad	1450102	Model TC20; for counting cells
Pierce™ BCA Protein Assay Kit	Thermo Scientific	23225	for measuring protein concentration in cell lysates
Cell lifter	Fisher	07-200-364	to remove cells from dish
Lysis Buffer (1 L)			20 mL 1 M Tris pH 7.4 10 mL glycerol 30 mL 5 M NaCl 840 mL ddH ₂ O 10 mL Triton-X100 (protease and phosphates inhibitors as desired)
10X TAE (1 L)			48.4 g Tris base

			11.42 mL glacial acetic acid 20 mL 0.5M EDTA pH 8 ddH ₂ O to 1 L
4X SDD-AGE loading buffer (50 mL)			5 mL 10X TAE 10 mL glycerol 4 mL 20% SDS 0.5 mL 10% bromophenol blue 31 mL ddH ₂ O
TBS Transfer Buffer (1 L)			20 mL 1 M Tris pH 7.4 30 mL 5 M NaCl 950 mL ddH ₂ O
PBST Wash Buffer (1 L)			100 mL 10xPBS 800 mL ddH ₂ O 1 mL Tween20
10X PBS (10 L)			800 g NaCl 20 g KCl 144 g Na ₂ HPO ₄ ·2H ₂ O 24 g KH ₂ PO ₄ add ddH ₂ O to 10 L