Notes:

∗ Chlamydomonas: I run samples on a 4% stacking, 10% running PAGE gel. 1.5 mm thickness. 10 well combs (for both Hoefer or Biorad minigels).
∗ Arabidopsis: I run samples on a 4% stacking, 15% running PAGE gel. 1.5 mm thickness
∗ Running buffer is regular PAGE buffer (14.4 grams glycine, 3 grams Tris Base).
∗ I also do not use ANGEL buffer solutions but instead use the same Tris solutions as for SDS-PAGE gels (1.5 M Tris-HCl pH 8.8 and 0.5 M Tris-HCl pH 6.8).

GELS

Running gel (**15%**)	for 10 mL	for 20 mL
1.5M Tris-HCl pH 8.8	2.5 mL	5 mL
acrylamide 40%	3.75 mL	7.5 mL
H_2O	3.75 mL	7.5 mL
10% APS	50 µL	100 µL
TEMED	9 µL	18 µL

Running gel (**10%**)	for 10 mL	for 20 mL
1.5M Tris-HCl pH 8.8	2.5 mL	5 mL
acrylamide 40%	2.5 mL	5 mL
H_2O	5 mL	10 mL
10% APS	50 µL	100 µL
TEMED	9 µL	18 µL

Stacking gel (**4%**)	for 5 mL	for 10 mL
0.5 M Tris-HCl pH 6.8	1.25 ml	2.5 ml
acrylamide 40%	0.5 ml	1 ml
H_2O	3.25 ml	6.5 ml
10% APS	25 µl	50 µl
TEMED	5 µl	10 µl

BUFFERS

Electrophoresis buffer (keep at 4ºC)
3 grams Tris Base.
14.4 grams glycine (192 mM glycine final concentration).

10X sample loading buffer (keep at -20 C)
250 mM Tris-HCl pH 6.8
50% Glycerol
0.02% bromophenol blue

Phosphate buffer (Kpi, pH 7.8)
90.8 ml K_2HPO_4 1M + 9.2 ml KH_2PO_4.
Add 100 mL H2) for 50 mM final concentration.

30 µM riboflavin
Prepare stock by weighing 11.3 mg/mL and resuspending in H_2O.
Take 50 µL from stock and add to 50mL 50mM Kpi pH 7.8.

0.48 mM NBT

Weigh 39 mg NBT and resuspend in 1 mL H_2O. As an alternative, use ready-made NBT pellets (Sigma # N5514). Dissolve one pellet (25 mg) in 100 mL KPi pH 7.8. Add 400 µL to 50 mL 50mM Kpi pH 7.8

PROTEIN EXTRACTION

+ spin down 1 mL culture, or 10^7 cells (room temp, 30 seconds, max speed)
+ resuspend in 100 µL 10 mM phosphate buffer.
+flash-freeze pellet in liquid nitrogen; let stand in liquid N_2 for 2 minutes.
+transfer tubes to metal blocks and allow to thaw for 2 minutes.
+vortex each tube for 5 seconds.
+repeat steps 3-5 one or two more times.
+spin samples down for 2 minutes at 4ºC, 12,000 rpm.
+quantify protein by BCA assay. Load 10-60 µg protein (less if using combs with more than 10 teeth; more protein means stronger signal, but lower resolution).

+take out 10-15 µL for supernatant containing soluble proteins, add 2-3 µL sample loading buffer.

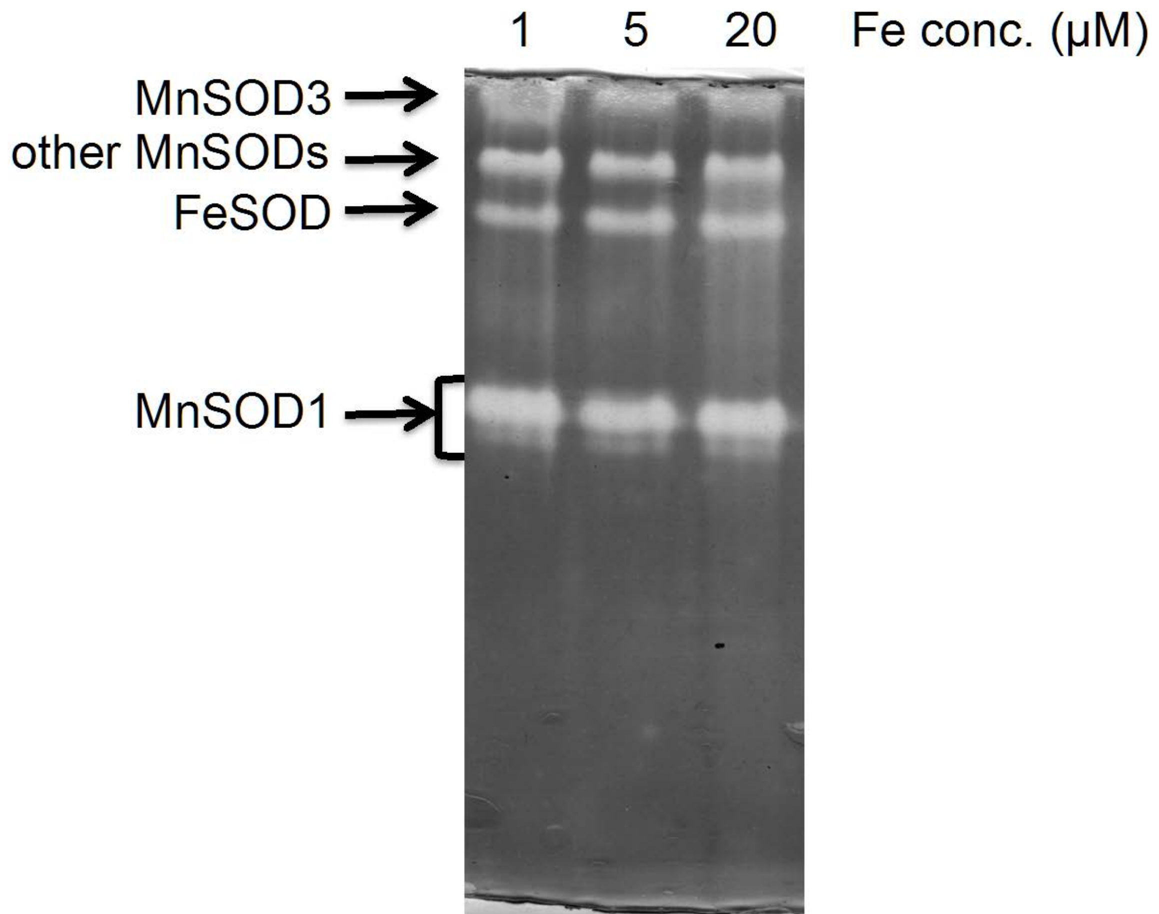
+run gels in the cold room, constant voltage, 200 V, for 2 hours. The loading dye will run off, but that’s fine.

STAINING

All steps are carried out at room temperature.
+incubate gels for 30 minutes in 40-50 mL 50mM Kpi pH7.8 in the dark. No shaking.
+incubate gels for 30 minutes in the dark with 0.48 mM NBT in 40-50 ml 50 mM KPi pH 7.8. Gentle shaking (50 rpm).
+one quick rinse with dH_2O.
+incubate for 30 minutes in the dark with 30 µM riboflavin 40-50 mL 50mM KPi pH 7.8. Gentle shaking (50 rpm).
+one quick rinse with dH2O.
+place gel on a glass plate and expose onto white light box until staining is sufficient.

H_2O_2 SENSITIVITY TEST

FeSOD are sensitive to H_2O_2 pre-treatment, but not MnSODs.
+run gels as described above.
+during the first incubation step in 50 mM Kpi pH 7.8, add 5 µL 30% H_2O_2 per 10 mL of KPi buffer used. Incubate for 30 minutes at room temperature in the dark, no shaking.
+quick rinse in dH_2O, then proceed to incubation with NBT solution.


1
5
20
Fe conc. (µM)
MnSOD3
other MnSODs
FeSOD
MnSOD1