NBT assay to measure reactive oxygen species.

Culture cells in 96-well plate and treat appropriately.
Remove media and wash cells with 200ul assay buffer.
Remove assay buffer and replace with 100ul reaction solution.
Incubate for 30 minutes in incubator. (This may need to be optimized.)
Remove reaction solution and wash with 200ul assay buffer.
Remove assay buffer and wash with 100% methanol.
Remove methanol and air dry.
Add 85ul 2M KOH.
Cover with foil and incubate on shaker for 10 minutes.
Add 100ul DMSO. (Do not remove KOH.)
Cover with foil and incubate on shaker for 10 minutes.
Determine absorbance at 540 and 630.

Assay buffer:
PBS + 1mM $CaCl_2$ + 1mM $MgCl_2$

Reaction solution:
Assay buffer + 0.04% NBT

Lysis solutions:
2M KOH
DMSO