Protein gels:

Reagents:

1 mL 4xLDS+BME

-1 mL 4x LDS sample buffer (Thermo NP0007)
-50 µL Beta-mercapto ethanol.
Mix in an Eppi, store at room temp for up to 2 months.

1 L 10x Tris-Glycine-SDS (TGS) Running buffer

-30 g Tris Base
-144 g Glycine
-10 g SDS
-ddH_2O to 1 L, store at RT

1 L 1x TGS running buffer

-100 mL 10x Tris-Glycine-SDS
-ddH_2O to 1 L, cool to 4^oC and store at 4^oC for up to 1 month and/or 3 uses

500 mL 1x MOPS-SDS running buffer

-25 mL 10x MOPS-SDS (Thermo NP0001)
-ddH_2O to 500 L, cool to 4^oC and store at 4^oC for up to 2 month and/or 6 uses

500 mL 1x MES-SDS running buffer

-25 mL 10x MES-SDS (Thermo NP0002)
-ddH_2O to 500 L, cool to 4^oC and store at 4^oC for up to 2 month and/or 6 uses

2 L Coomassie Brilliant blue stain

-1.5 g Coomassie blue R250 or G250
-800 mL Methanol
-200 mL Glacial acetic acid
-ddH_2O to 2 L, store at RT

1 L Destain solution

-100 mL Methanol
-100 mL Glacial acetic acid
-ddH_2O to 1 L, store at RT

Protocol:

Sample prep (for total protein gels stained with Coomassie)

-Prepare purified protein samples at 0.1-0.3 mg/mL in 1x LDS+BME (e.g. for a single protein at 1 mg/mL, add 4 µL protein, 26 µL ddH_2O, and 10 µL 4x LDS+BME and for a protein complex use higher concentrations.). Boil samples for 3-10 min and centrifuge at 20,000 x g for 10 seconds prior to loading.
-For bacteria, pellet the equivalent of 1 mL of A_{600} = 0.3 cells and resuspend in 75 µL lysis buffer (1% IGEPAL co 630, 1% Triton TX100, 1% Sodium deoxycholate, 0.1 mg/mL lysozyme added fresh prior to use). Incubate at room temperature for 10 min

then add 25 µL 4X LDS+BME. Boil samples for 3-10 min, sonicate for 5 min on high in the bath sonicatior, and centrifuge at 20,000 x g for 10 seconds prior to loading.
-For Insect cells, pellet 0.5e6 cells and resuspend in 75 µL water and 25 µL 4xLDS+BME. Boil samples for 3-10 min, sonicate for 5 min on high in the bath sonicatior, and centrifuge at 20,000 x g for 10 seconds prior to loading.
-**NOTE:** SYPRO Ruby is approx. 5x more sensitive than Coomassie, so use 2-5 fold less samples when running a gel that will be stained with SYPRO
-**NOTE:** Westerns are approx. 10-100x more sensitive than Coomassie, so use approx 10-100 fold less samples when running a gel that will be stained with SYPRO

Gel/buffer choice
-Consult manufacturer for gel choice. Typically, lower percentage gels are better at separating large proteins and higher percentage are better for smaller species.
-**NOTE:** For a given acrylamide percentage Bis-Tris gel, MOPS-SDS running buffer will work better for separating heavier proteins and MES-SDS will work better for smaller proteins. See https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-gel-electrophoresis/protein-gels/protein-gel-selection-guide.html?ef_id=Cj0KCQiA8ICOBhDmARIsAEGI6o1y4FzxsiERWl6G0pPBlA6IxtUTk6Mw4FyAjNeXTv4DJDvG-O-EemcaApYZEALw_wcB:G:s&s_kwcid=AL!3652!3!514630551272!!!g!!&cid=bid_pca_gel_r01_co_cp1359_pjt0000_bid00000_0se_gaw_dy_pur_con&gclid=Cj0KCQiA8ICOBhDmARIsAEGI6o1y4FzxsiERWl6G0pPBlA6IxtUTk6Mw4FyAjNeXTv4DJDvG-O-EemcaApYZEALw_wcB for more details

Gel loading and running
-Gently load 5-30 µL sample/well, depending on application (wedge-wells hold more than Nu-PAGE, lower well count means higher well capacity.)
-Load the same volume of sample in each well, otherwise there will be artifacts and your gel will be less interpretable.
-Use an appropriate MW ladder. I prefer Benchmark protein plus ladder (Thermo Cat 10747012) diluted 1:5 in 1xLDS for total protein gels and a 1:1 mixture of the lab's prestained ladder and SuperSignal (Thermo cat 84785) for Westerns. Note that Prestained ladders will not work with SYPRO Ruby stain.
-Add 500-800 mL of the appropriate running buffer prior to running.
-Run Gel at 180V limiting for 70-90 minutes with cold running buffer. Run-times will vary depending on the application, but I usually make sure that the dye-front has just entered the 'foot' of the gel. If the gel warms up too much it will begin to melt, so add additional cold running.

Coomassie Staining
-Remove the gel from its casing and place into an OWL staining box (Thermo GSB-3) containing 50-100 mL Coomassie blue stain (NOTE: do not use CBB contaminated boxes for other applications! Coomassie is very hard to get rid of)
-Microwave for 25 seconds (IMPORTANT: if it's boiling stop the microwave!)
-Shake at room temp for 5-10 min

-Pour the Coomassie stain back into its original container (it can be reused many times) and immediately replace with ~100 mL destain.
-Fold three Kimwipes into a cylinder and place into the container and shake for 1-3 hours
-Image gel in the alpha imager or using the scanner

SYPRO Ruby Staining
-Remove the gel from its casing and place into a clean OWL staining box containing 50-100 mL destain
-Shake at room temp for 5-10 min
-Decant destain and replace with 50 mL SYPRO Ruby stain (Thermo S12000)
-Shake at room temp for 30-60 min
-Pour the stain back into a separate container (it can be reused up to three times) and immediately replace with ~100 mL destain.
-Shake at room temp for 60 min to overnight.
-Image gel in the alpha imager using the UV-gel setting

Western Blot
-Follow the Western Blot Protocol starting at the Transfer step