IP/Western:

When cells are ready for collection, wash cells in 1X PBS.
Scrape cells in 1X PBS. I usually use 1 mL per p100 dish and 2 mL per p150 dish.
Pellet cells @ 1500 rpm, 2', 4°C (alternatively, 1000 rpm, 5', 4°C) in a centrifuge that holds 15 and 50 mL conical tubes (usually for tissue culture). I usually use the faster speed if I know I will have a lot of cells. If using a microfuge, I pellet cells @ 3000 rpm, 2', 4°C.
Lyse cells on ice in RIPA buffer (for senescent BJs) containing protease inhibitors. (When I evaluated the senescent BJs, I used about 200 uL RIPA buffer for 2 p150 dishes of harvested senescent cells. I used the same volume of RIPA for 1 p150 dish of harvested young BJs.)
Pipette gently up and down several times to break up the cells. At this time, I transfer the lysates to eppendorf tubes to lyse on ice.
After about 15', sonicate samples at output level 6, 30 second bursts with 30 second rests in-between for 3' (as recommended on Sheila's sonicator).
Centrifuge samples @ 14,000 rpm, 3', 4°C. Save supernatant (lysate).
Determine protein concentration. Calculate and adjust volumes so that all IP reactions have equal amounts of proteins and volumes. Save 10% of the total lysate volume for direct western blotting and store @ 80°C.
For each IP, I usually use 1 ug of antibody for every 100 ug of protein. If the antibody is not already agarose conjugated, then add 50 uL of protein A/G-Plus agarose to the samples. Include an IgG control IP reaction. Rock samples overnight at 4°C.
Pellet beads @ 3000 rpm, 3', 4°C.
Wash beads in RIPA buffer (400-500 uL) twice. I usually invert the tubes gently back and forth as I am walking to the cold room to centrifuge. During the last wash, save about 20-30 uL of buffer on top of the pelleted beads.
Add 6x loading dye (usually 6-7 uL) to each sample and finger flick the tubes to mix.
Heat @ 95°C for 5'. Pulse spin the samples (a few seconds to bring everything down).
Resolve the proteins in a 6% SDS-polyacrylamide gel (for Rb). Run at 100 volts until the 75 kD marker is at the bottom of the gel.
Transfer proteins onto nitrocellulose in 1X Towbin buffer, overnight, 4°C, @ 38 volts.
Block membrane with 5% non-fat dry milk in 1X TBST for one hour at room temp.
Probe with primary antibody (for Rb, I use IF8 from Santa Cruz (sc-102) at 1:500 dilution) in 1X TBST-milk overnight, 4°C.
Wash membrane in 1X TBST twice, 15' per wash.
Probe with secondary antibody for 1 hour at room temp.
Wash membrane in 1X TBST twice, 15' per wash.
Visualize protein bands using ECL Plus Western Blotting Detection Reagents (Amersham).

Antibodies:

For Rb, it's a good idea to IP Rb first and then probe for Rb since it is difficult to detect Rb by western blotting directly. For the IP, I use the IF8 agarose-conjugated antibody from Santa Cruz (sc-102AC). Both the hyperphosphorylated and hypophosphorylated forms should be visible from the 6% SDS-PAGE.
For p53, I haven't had to look at phosphorylated p53, but for total p53, I use DO-1 from Santa Cruz (sc-126) at 1:500 dilution.

For p21, I use C-19 from Santa Cruz (sc-397) at 1:500 dilution. For p53 and p21, I have been able to detect both proteins in the BJs by western blotting directly, but in response to DNA damage and not during senescence so the lysis buffer is a little different (IPH lysis buffer – see below for recipe). Usually I use a 4-20% pre-cast gel and run out my 10% input sample (see above) and cut the blot to probe for the appropriate proteins at the same time. If I'm using the pre-cast gel, I will transfer proteins overnight onto nitrocellulose at 20 volts, 4°C.
For most proteins unless they are abundantly expressed in the cells, I incubate my blots in primary antibody overnight. For example, for our loading control, we use tubulin and this I can incubate for 2 hours at room temp in primary antibody (C-20, from Santa Cruz (sc-7396)).

Buffers:

RIPA
150 mM NaCl
1% NP-40
0.5% NaDeoxycholate
0.1% SDS
50 mM Tris, pH 8.0

IPH
50 mM Tris, pH 8.0
150 mM NaCl
0.5% NP-40
5 mM EDTA

10X TBST
250 mM Tris, pH 8.0
1.25 M NaCl
0.5% Tween 20

1X Towbin (1 Liter)
3.0g Tris
14.4g Glycine
200 mL Methanol