Soft Agar Assay Protocol

1. Preparation of Base Agar:
    a. Dissolve 1% agarose (Difco Agar Noble) in sterile H_20, cool to 42°C in water bath. Use autoclaved 125 mL screwtop bottle
    b. Warm 2X DMEM w/20% FBS and antibiotics to 42° in water bath.
        i. It's best to equilibrate a 50 mL tube of DMEM and a 125 mL bottle of soft agar together in a water-filled 500 mL glass beaker within the water bath, so that they fit snugly and can be transported into the hood and kept at 37°C longer.
        ii. 2X DMEM recipe: 38 mL 2X straight DMEM, 10 mL FBS (20%), 1 mL L-Glut (2X), 1 mL antibiotic (2X). I just used concentrations of FBS, L-Glut, and antibiotic that were already made up.
    c. Equilibrate for about 45 minutes.
    d. Bring beaker with DMEM and agarose into the hood.
    e. Mix equal volumes of the agarose and the 2X DMEM to give 0.5% agarose/1X media. Also add appropriate amount of G418 if necessary.
    f. Add 1 mL of mixture from step C to 6-well dish (1 mL/dish), tap sides of plate to spread evenly, allow 5 minutes to solidify.
        i. Plates can be stored in 4° C for up to one week.

2. Preparation of Top Agarose:
    a. Bring out Base Agar plates to equilibrate to room temperature if not already.
    b. Dissolve 0.7% agarose in sterile H_2O), cool to 37°C in water bath with 2X DMEM in the same manner as the base layer.
    c. Count cells
        i. Concentrations of cells I used were 2500, 7500, 20,000.
    d. Add ½ media, ½ agarose solution. Make sure to add cells and G418 before adding the agarose, since the agarose quickly solidifies.
    e. Add 1 mL to each well of the base agar layer, allow 15 minutes to solidify.
    f. Add 1 mL 1X full media + G418 to each well.
    g. Incubate plates at 37°C in humidified incubator 10-30 days (I did this over 14 days).
    h. Feed cells twice per week.

3. Staining:
    a. Stain cells with 0.5 mL Crystal Violet + methanol for >1 hour.
    b. Wash Crystal violet off by adding 1-2 mL water to soft agar 4-5 times.
    c. Image on a dissecting microscope.
    d. Count colonies > 500 μm or other appropriate diameter. Easiest to use ImageJ → Binary → Analyze Particles if there are a lot of colonies.