by centrifugation at 250*g*. Resuspend in 10 mL of PBS and perform a viable cell count (*see* **Note 9**).

Harvest the myeloma cells from the tissue culture flasks by removing the medium and replacing it with PBS containing 0.02% (w/v) ethylenediaminetetra-acid (EDTA). Once they have detached from the surface of the flasks decant into universal containers and wash by centrifugation at 150*g*. A viable cell count should be performed to estimate numbers (*see* **Note 9**).

3.2.4. Cell Fusion (Modification of ***ref.*** **8***)*

Keep all media in a water bath or incubator set at 37°C.

1. Place 26 × 10^6 each of spleen and myeloma cells together in a universal container.
2. Pellet cells at 125*g* for 5 min and pour off PBS.
3. Tap pellet gently to loosen cells.
4. Add 1 mL of 50% polyethylene glycol 4000 (Fisons, Leicestershire, UK) (PEG)/RPMI-1640 medium (no FBS).
5. Resuspend cells gently.
6. Pellet cells at 175*g* for 5 min.
7. Add 5 mL of RPMI-1640 medium (no FBS) do not disturb the pellet.
8. Gently resuspend.
9. Pellet cells at 175*g* for 5 min.
10. Pour off supernatant and slowly add 5 mL HAT medium with 15% FBS.
11. Leave pellet for 7 min, then gently resuspend.
12. Place 0.1 mL of HAT medium with 15% FBS and 15% MTM medium into each of the 96 wells of a tissue-culture plate. Add 0.1 mL of the cell suspension from **step 11**.
13. Incubate the cells in a 37°C incubator with 5% CO_2 and 90% humidity for 7 d, and then assess visually for colony growth. Each well should have 0.1 mL of HAT medium with 15% FBS added. When cell colonies are one-third to one-half confluent in the wells, the tissue culture supernatant should be assayed (*see* **Notes 2** and **3**) for the presence of the antibody of interest (*see* **Subheading 3.5.**; *see* **Note 10**).
14. Cells that are producing the desired antibody should be cloned by limiting dilution (1 cell/well) until stability and clonality can be ensured.
15. Keep stocks of cells in liquid nitrogen so that MAbs can be produced at a later date.
16. Bulk cultures of cells can be grown to produce mg quantities of MAb. Cell lines produce 10–50 mg/L of medium, which can then be purified by affinity chromatography on protein A or G (Pharmacia, St. Albans, UK).

3.3. Purification of PAb

In **steps 2–3**, work in a small, clean beaker on a magnetic stirrer set at a slow speed at room temperature.