

Liquid Subculture: BY2 cell suspension

| Volume | 1L | Notes |
|--|-----|-----------------------------|
| MS Salt (no vitamins) (g) | 4.3 | 1 packet MS |
| Sucrose (g) | 30 | |
| 500x Myo-Inositol (ml)[0.1g/L]** final concentration | 2 | Autoclave and store at 4 C |
| 500x Thiamine Solution [1mg/L] | 2 | |
| 500x 2,4 D Solution [0.2mg/L] | 2 | Add KOH or EtOH to dissolve |
| 500x KH ₂ PO ₄ [200 mg/L] | 2 | |

- pH to 5.8 with 1M KOH (from 10M KOH)
- autoclave 300 ml or 500 ml flask and aliquot solution 100ml, cover with foil and autoclave
- subculture 1 ml weekly, or 4-5ml every four days.
- Add antibiotics dependent on selective marker (i.e. 100ul of 100 mg/ml Kan or 20 ug/ml Hygromcin (final concentration))
- Add phytigel -3g/L to make plates for callous

BY2- Agro Transformation/Wash continue after electroporation (use 4-5 out of 7 day culture)

1. Transform Agro by electroporation, select single colonies, and grow 2-3 days at 28C
2. Co-culture 2 days (1, 10 or 100 ul of Agro w/ 10-15 ml of cells)
3. Pipet BY2-Agro solution with 10 ml media for easier pipetting (or 8 ml in square plate) -cover bottom, seal edges with parafilm, cover in foil at RT 2 days
4. Add BY-2 media to 40 ml after cell transfer to 50 ml conical falcon tubes
5. Spin 8000 rpm for 3 min with 9 acceleration and 5 brake
6. Wash 5x (fill up the 50 ml conical) and decant
7. 3 ml per plate (5 per strain) before that add 1000x Carb (1ul/10ml or 100mg/ml)
8. Break tip for larger suction
9. Spread uniformly on plate
10. Pick out large colony after 21 days or so and transfer like normal cells from this point. The colony should not be either hard and crunchy or squishy. You can screen several cells early (within 1.5 weeks) to pick positive fluorescent clones.

| Ingredients | Final amount (mg/L) |
|---------------------------------|---------------------|
| Macronutrient | |
| KNO ₃ | 1900 |
| NH ₄ NO ₃ | 1650 |
| KH ₂ PO ₄ | 170 |
| Micronutrient | |
| KI | 0.83 |
| H ₃ BO ₃ | 6.2 |

| | |
|---|-------|
| MnSO ₄ -5H ₂ O | 24.1 |
| ZnSO ₄ -7H ₂ O | 8.6 |
| Na ₂ MoO ₄ -2H ₂ O | 0.25 |
| CuSO ₄ -5H ₂ O | 0.025 |
| CoCl ₂ -6H ₂ O | 0.025 |
| Macronutrient | |
| CaCl ₂ -2H ₂ O | 440 |
| MgSO ₄ -7H ₂ O | 370 |
| Fe(III)-EDTA | 42.1 |
| Others | |
| Sucrose | 30000 |
| Myo-Inositol | 100 |
| Thiamine-HCl | 1 |
| 2,4D | 0.2 |
| KH ₂ PO ₄ | 200 |