

ONPG preparation (4mg/ml)

Phosphate buffer:

To prepare 50ml of 0.2M Phosphate buffer at pH 7.2 mix:

- 36ml $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 0.2M
- 14ml $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.2M

| pH, 25 deg. C | x ml 0.2M Di-sodium | y ml 0.2M mono-sodium |
|---------------|---------------------|-----------------------|
| 5.8 | 4.0 | 46.0 |
| 6.0 | 6.15 | 43.85 |
| 6.2 | 9.25 | 40.75 |
| 6.4 | 13.25 | 36.75 |
| 6.6 | 18.75 | 31.25 |
| 6.8 | 24.5 | 25.5 |
| 7.0 | 30.5 | 19.5 |
| 7.2 | 36.0 | 14.0 |
| 7.4 | 40.5 | 9.5 |
| 7.6 | 43.5 | 6.5 |
| 7.8 | 45.75 | 4.25 |
| 8.0 | 47.35 | 2.65 |

Original reference;

Gomori, after Sorensen, Meth. Enzymol. 1, 143 (1955)

1. Add 50ml DDW to 50ml 0.2M Phosphate Buffer to bring to a concentration of 0.1M
2. Add 62.5ml 0.1M Phosphate Buffer pH 7.2 to 250mg ONPG (Sigma N1127) and gently warm with stirring in order to completely dissolve the product at a concentration of 4mg/ml.
3. Aliquot and freeze at -20C.