

|                   | Stock concentration | Final concentration | Volume      |
|-------------------|---------------------|---------------------|-------------|
| Reaction volume   | -                   | -                   | 300 $\mu$ L |
| 5 $\times$ buffer | 5 $\times$          | 1 $\times$          | 60 $\mu$ L  |
| rNTP mix          | 25 mM               | 5 mM                | 60 $\mu$ L  |
| RNase inhibitor   | 40 U/ $\mu$ L       | 0.7 U/ $\mu$ L      | 5 $\mu$ L   |
| Pyrophosphatase   | 100 U/mL            | 1.7 mU/ $\mu$ L     | 4 $\mu$ L   |
| DTT               | 100 mM              | 3.3 mM              | 10 $\mu$ L  |
| T7 RNA polymerase | 50 U/ $\mu$ L       | 3.3 U/ $\mu$ L      | 20 $\mu$ L  |
| Template          | 120 ng/ $\mu$ L     | 2 ng/ $\mu$ L       | 5 $\mu$ L   |
| Water             | -                   | -                   | 136 $\mu$ L |