QUICK PROTOCOL

# Ovation® WGA System (6100-12)

Enter the following information to automatically calculate the volumes needed to prepare each reaction. Simply print this page and record the essential information for this experiment.

Operator's Name: _____ Date: _____

Ovation WGA System Lot No. _____ RNAClean® Bead Lot Number _____

SPIA Product Purification Kit Name and Lot No. _____ Number of Samples _____

| Thermal Cycler Programming | |
|---|---|
| PROGRAMMING DETAILS | |
| **Program 1:** Denaturation | 95°C for 3 min<br>4°C forever |
| **Program 2:** Template Synthesis | 4°C for 1 min,<br>[ 23°C for 10 min, 35°C for 10 min, 57°C for 10 min] **X 2**,<br>70°C for 5 min<br>4°C forever |
| **Program 3:** SPIA Amplification | 4°C for 1 min<br>47°C for 90 min<br>95°C for 5 min<br>4°C forever |

| Denaturation and Template Synthesis | | | | |
|---|---|---|---|---|
| Thaw the **Denaturation and Template Synthesis Reagents (blue)** and **Nuclease Free Water (green)**. Mix each reagent, spin and place on ice | | | | |
| Add 2 µL of **DA1** into 0.2 mL PCR tube and place on ice | | | | |
| For each sample add 8 µL of gDNA (10-50 ng), flick tubes to mix and spin | | | | |
| Place the tubes in a thermal cycler running **Program 1** | | | | |
| Immediately remove tubes from the thermal cycler after 95°C step and place tubes on ice for 5 min to snap cool | | | | |
| Prepare **Template Synthesis Master Mix** (10% overfill is included in the calculation) | **No. of Samples** | **DA2** | **DA3** | **DA4** |
| | 1 | 2 µL | 7 µL | 1 µL |
| | 0 | 0 µL | 0 µL | 0 µL |
| Spin **Template Synthesis Master Mix** and place on ice | | | | |
| Add 10 µL of the **Template Synthesis Master Mix** to each tube, mix and spin | | | | |
| Place the tubes in a thermal cycler running **Program 2** | | | | |

## Purification of Template cDNA

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| Bring the RNAClean beads to room temperature and resuspend |
| Add 32 µL of beads to each reaction and mix |
| Incubate at room temperature for 10 min |
| Place tubes on magnet for 5 min to completely clear the beads |
| To minimize bead loss, remove only 40 µL of Binding Buffer before the first wash step |
| Wash the beads while still on magnet for 30 sec with 200 µL of freshly prepared 70% ethanol |
| Repeat wash 2 more times |
| Dry beads completely, at least for 15-20 min |
| Proceed immediately to SPIA® amplification. |

## SPIA Amplification

Thaw the **SPIA Reagents (red)**. Vortex **DB1** and **DB2**. Invert **DB3** 5 times, spin and place on ice.

Prepare **SPIA Master Mix** (10% overfill is included in the calculation)

| No. of Samples | DB2 | DB1 | DB3 |
|---|---|---|---|
| 1 | 20 µL | 10 µL | 10 µL |
| 0 | 0 µL | 0 µL | 0 µL |

| |
|---|
| Mix **SPIA Master Mix**, spin and place on ice |
| On ice, add 40 µL of **SPIA Master Mix** to each template synthesis reaction tube containing the dried beads, mix |
| Place tubes in a thermal cycler running **Program 3** |
| Once the thermal cycler reaches 4°C, spin and place tubes on ice |

**Note:** Complete the remainder of the protocol in the Post-Amplification area

## Purification of Amplified SPIA Product

Refer to the user guide and follow your method of choice for purification. Document the protocol below for future reference.

| | | |
|---|---|---|
| If not using RNAClean Bead method for the final SPIA product cleanup, place tubes on magnet plate and let stand for five min before transferring the cleared supernatant containing SPIA product to a fresh tube. Discard the beads | | |
| Proceed immediately to purification step or store SPIA product at -20°C | | |
| Add Binding Buffer in volume of: | Spin at speed: | For a duration of: |
| Add Wash Buffer in volume of: | Spin at speed: | For a duration of: |
| Repeat for second Wash. | | |
| To elute sample use **Nuclease-Free Water D1** provided with the Ovation WGA System | | |
| Add **Nuclease-Free Water D1** in volume of: | Spin at speed: | For a duration of: |

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