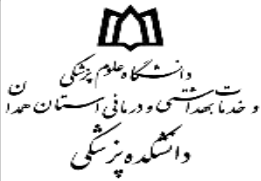


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Standard Operating Procedure (SOP): Real-Time PCR (StepOnePlus™)

1. Objective

To define the operational procedure for the Applied Biosystems™ (ABI) StepOnePlus™ Real-Time PCR system for accurate DNA and RNA quantification and analysis.

2. Scope

This equipment is used for high-sensitivity detection and measurement of nucleic acids, including:

- Gene expression analysis.
- Pathogen detection.
- Allelic discrimination.
- Mutation analysis (insertions/deletions/SNPs).
- Quantitative template assessment (e.g., telomere length measurement, mitochondrial DNA copy number counting).

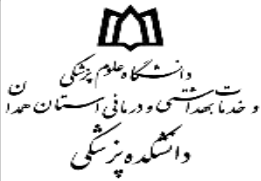
3. Responsibility

The responsibility for implementing this procedure lies with the designated laboratory experts.

4. Operational Procedure

1. **Power Up:** Turn on the device using the main power switch located on the rear panel.
2. **Software Setup:** Open the StepOne™ software on the connected laptop and select **Advanced Setup** to create a new experiment.
3. **Experiment Configuration:** Define the experiment type in the **Experiment** properties menu.
4. **Plate Setup:**
 - Navigate to the **Plate Setup** tab.
 - Use the **Define Target** sub-tab to specify the reporter dyes.
 - Use the **Assign Target** sub-tab to map the samples to their specific wells.
5. **Thermal Protocol:** In the **Run Method** tab, define the cycling conditions (thermal protocol), sample volume, and total number of cycles.
6. **Save:** Click **Save** to store the experiment file.
7. **Execution:** Click the **Start Run** button to initiate the PCR program.
8. **Power Down:** Once the run is complete, shut down the device using the power switch on the rear panel.

تاریخ اجرا:	تصویب کننده:	تأیید کننده: دکتر محمدرضا عربستانی	تهیه کننده: حسین فضلی
تاریخ بازنگری:	سمت:	سمت: مدیر گروه میکروبیشناسی	سمت: سوپر وایزر آزمایشگاه
شماره صفحه: ۱ از ۲	تاریخ و امضاء:	تاریخ و امضاء:	تاریخ و امضاء:

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5. Quality Assurance and Validation

- **Positive/Negative Controls:** Must be included in every run. Use a known positive template and a No-Template Control (NTC) to ensure assay validity.
- **No-Enzyme Control:** Use a control without polymerase to verify that observed fluorescence is not due to non-enzymatic artifacts.
- **Annealing Temperature:** Optimize and set the annealing temperature precisely to prevent non-specific amplification.
- **Melt Curve Analysis:** Always analyze the melt curve (dissociation curve) post-amplification to confirm product specificity and detect potential primer-dimer formation.
- **Technical Notification:** If the system exhibits errors or requires non-standard parameter adjustments, contact the technical supervisor immediately.

6. Safety Guidelines

- **General Safety:** Real-Time PCR safety involves protecting the operator, ensuring assay integrity, and preventing cross-contamination.
- **PPE:** Always wear appropriate personal protective equipment (PPE), including **gloves, lab coat, and face mask** when handling biological samples.
- **Laboratory Hygiene:** Maintain a clean work environment and adhere to strict molecular biology practices to prevent template contamination.

7. Maintenance and Care

- **Power Protection:** The device **must** be connected to a stabilizer and an Uninterruptible Power Supply (UPS) to protect sensitive electronics from power surges and outages.
- **Well Maintenance:** If any liquid, residue, or ink marks are found in the sample wells after a run, clean them immediately using a soft cloth or a swab lightly dampened with 70% ethanol. Ensure the wells are completely dry before the next use.

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