

| **PREP**   * Pen & sharpie labeled with green tape * Separation of Post-Amp area * Heaters on (Heat indicator lit!) * Safety Procedures: * lab coat * gloves * face mask * face shield or goggles * Alcohol wipe sample tubes |  | Log Link: SOP-104-B\_1 {SITE} | |
| --- | --- | --- | --- |
| Location: | |
| Name: | | |
| Date:  Time: | | |

**CONTAMINATION INFRACTIONS**

* PPE lapse/removal
* Use green labeled pen/marker with bare hands and not putting it to be cleaned afterwards
* Use unmarked pen/marker with clean gloves (without cleaning gloves after or changing gloves)
* Bare hands on anything improper, except in desk area
* Reuse pipette tip improperly
* Touch pipette tip to anything and then not discard it
* Touch anything dirty then go back to work (face, mask, uncleaned phone, uncleaned laptop)

# **NOTE**

It is recommended that 1XISS and Reaction Mix be prepared before the assay run (instructions below).

**DOCUMENTATION**

* Check off each item after completing it
* Write legibly

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# **GLOSSARY OF TERMS**

**100XIS**………………………….. 100-fold concentrated Inactivation Solution

**1XISS**……………………………. 1X Inactivation Saline Solution

**PGS**………………………………. Primer Guanidine Solution

**CLAMP MM**…………………. Colorimetric Loop-Mediated Isothermal Amplification Master Mix

**RM**………………………………… Reaction Mix (CLAMP MM and PGS)

**TPC**………………………………. Twist Positive Control

| **1XISS PREP**   * Record volumes of saline & 100XIS in 1XISS Prep form prior to pipetting * Combine saline and 100XIS in a 50mL falcon tube or 30mL Chub tube, depending on volume * Vortex 30 seconds * Label tube with 1XISS ID (e.g., “1X\_MMDD-1”) and record the same ID into the 1XISS Prep Form and the table on this form | Sample Batch ID (from App): | | |
| --- | --- | --- | --- |
| Inactiv Heater: | | |
| 1X Inactiv Saline Soln ID: | | |
| Dispense: Pipette or Manual Disp or Electric Disp | | |

**INACTIVATION**

* Add 1mL of 1XISS to each sample tube
* Verify that tubes are properly sealed afterward (i.e., not cross-threaded)
* Vortex 10 sec (if individual tubes) or 30 sec (if tubes-in-rack)
* Heat 8 min in Water Bath (set to 99°C)
* Cool 10 min
* Put in refrigerator before 30 minute mark

**REACTION MIX PREP**

* Complete the entry in the RM Prep Form prior to pipetting
* Calc volumes correctly for PGS and CLAMP based on number of reactions
* Remove CLAMP MM and PGS from freezer to thaw, but do not allow them to warm
* Briefly spin down reagent tubes (removes reagent from the cap and tube walls)
* Pipette CLAMP MM and PGS into a 1.5mL tube using calculated volumes
* Promptly return PGS and CLAMP source tubes to freezer
* Vortex and spin down the RM tube
* Aliquot 23uL of RM into each reaction tube (plate or strips-of-8)
  + Pipetting at the bottom of tubes
  + No blowout while dispensing reaction mix (to prevent bubbles in bottom of well)
* Visually check the volumes in each reaction tube (that they appear to be even)
* If using a plate, cover filled tubes with rack lid to prevent dust
* At this stage, RM reaction tubes can be capped and placed in the freezer for storage if they are not to be used fresh.

| **AMPLIFICATION REACTION**   * Remove PCR cold block from freezer ~10 min before adding tubes * Prep RM plate/strips (thawed, not warm) * Set up tips to align with reactions * Use a lookup rack to keep track of sample order * Tip layout aligns with arrangement of sample tubes in lookup rack & aligns with layout of reaction plate/strips * Add negative control first (2uL 1XISS) * Add 2uL of each inactivated sample to reaction tubes, visually checking the volume in the tip each time. Pipette up & down 5X, blow out in liquid, tip touch. | Amp Heater: | | Strip8 Tubes or Plates |
| --- | --- | --- | --- |
| Reaction Mix: Frozen or Fresh | | |
| Reaction Mix ID: | | |
| Num Reactions (including controls): | | |
| Pos Ctrl ID: | | |
| Neg Ctrl ID (today's 1XISS): | | |
| Initial Inconclusives?: | | |
| * With each add, verify the correct position (tip vs sample tube vs reaction tube) * Transfer plate/strip to PCR rack (not cold block - rxns don’t go to amp cold) * Retrieve TPC from freezer, touching only with one hand (consider this hand dirty) * Add 2uL TPC to pos. ctrl, return TPC to freezer (IMMEDIATELY change glove) * Cap reaction tubes / seal plate * Change gloves again after loading tubes/plate onto amp heater * Set main timer for 25 min * Set secondary timer for 24 min * Put sample tubes in fridge * Remove rxns from amp promptly, wait 1 minute before imaging * Take photo in lightbox * For photo apply crop and ‘Vivid’ filter (or increase saturation 70% for android) * Log amp run sheet with QR code * On app: Intake, Process and Result tubes | Notes: | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** |
| **A 1** |  |  |  |  |  |  |
| **B 2** |  |  |  |  |  |  |
| **C 3** |  |  |  |  |  |  |
| **D 4** |  |  |  |  |  |  |
| **E 5** |  |  |  |  |  |  |
| **F 6** |  |  |  |  |  |  |
| **G 7** |  |  |  |  |  |  |
| **H 8** |  |  |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **7** | **8** | **9** | **10** | **11** | **12** |
| **A 1** |  |  |  |  |  |  |
| **B 2** |  |  |  |  |  |  |
| **C 3** |  |  |  |  |  |  |
| **D 4** |  |  |  |  |  |  |
| **E 5** |  |  |  |  |  |  |
| **F 6** |  |  |  |  |  |  |
| **G 7** |  |  |  |  |  |  |
| **H 8** |  |  |  |  |  |  |