BioCode® GPP

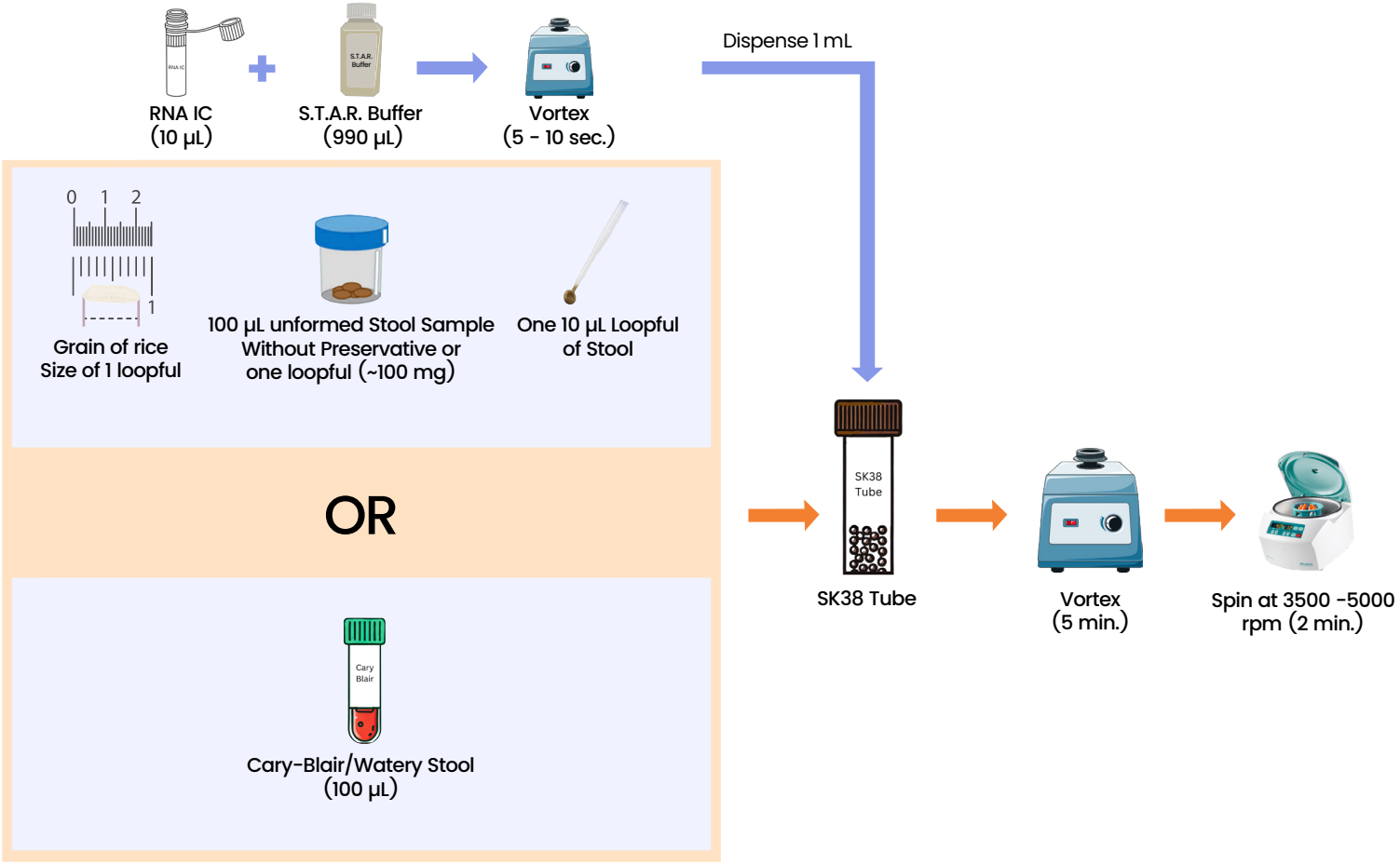
Tips for Successful Sample Prep and Extraction With KingFisher™ Flex

Stool Amount:

Mix RNA IC and S.T.A.R. Buffer at a 1/100 ratio (v/v) to obtain 1 mL of solution for each specimen plus a negative control. Vortex for 5-10 seconds, then dispense into the SK-38 tubes. **Add 100 µL Cary-Blair** or watery stool or one loopful (~100 mg) of formed stool to the SK38 tubes. Use 10 µL-Loop to pick up a loop of formed stool to add to SK38 tube. For the negative control, add 100 µL clean media (i.e. S.T.A.R. Buffer) or well characterized negative sample. Do not add more stool than instructed. Doing so may lead to "**invalid results**".

Note:

A valid negative control is **required** for each plate/kit lot to obtain results.



Extraction:

KingFisher™ Flex

Instrument Prep:

- Label 5 96 deep well plates **Wash 1, Wash 2, Wash 3, Sample**, and **Tip Comb**. Label 1 96-well microplate **Elution**.
- Place a tip comb for 96 deep well plates in the "Tip Comb" plate.
- Add **500µL** of Wash Solution from kit to each sample-processing well in the "Wash 1" plate.
- Add **500µL** of Molecular Grade 80% Ethanol to each sample-processing well in the "Wash 2" plate.
- Add **250µL** of Molecular Grade Ethanol to each sample-processing well in the "Wash 3" plate.
- Add **60µL** Elution Solution from kit to each sample-processing well in the "Elution" microplate.
- Cover the reagent plates until ready to load, to prevent evaporation.

Sample Prep:

- Vigorously vortex Binding Beads for at least 30 seconds. Prepare Binding Solution by mixing 265µL Binding Buffer with 10µL Binding Beads in a sterile tube for each sample processed, plus 10% overage. Mix by inversion only, do not vortex. Binding solution is stable at room temperature for 30 min.
- Using a repeater pipette, add 10µL proteinase K to each sample well.
- Add 200µL of SK-38 processed sample to each well, pipette up and down to mix thoroughly.
- Add 275µL of the prepared Binding Buffer Solution to each well, pipette up and down to mix thoroughly.

Perform Protocol: ABC_GPP_KFF_v1.bdz

Enter lot information prior to loading.

- Load all plates onto the instrument, following the onscreen prompts from the unit.
- When the runs are complete, store the elution plate and place all other reagents in a plastic bag to dispose of in a biohazard bin.

Nucleic Acid Storage Conditions:

Optional: Transfer sample extracts from the plate into PCR grade container.

2-8°C refrigerator

If testing **within** 24 hours.

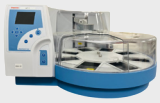
80°C or below

If testing **cannot** be completed within 24 hours of extraction.

Note

- Store extracted nucleic acids at -80°C or below for up to 90 days.
- Store leftover pretreated samples (in SK38 tubes) at -80°C or below for up to 90 days.

Repeat/Reflex Extraction:



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- Transfer **50 µL** from the SK38 tube and 150 µL S.T.A.R. buffer into the same plate.
- **Perform Protocol:** Proceed with same protocol as above.

Address 12130 Mora Dr., Unit 2 Santa Fe Springs, CA, 90670 USA
Phone 1-833-262-8324
Website www.apbiocode.com
E-mail techsupport@apbiocode.com