Sansure Ultra HBV Manual Extraction and Amplification SOP

Notes before start:

☐ Go through Sansure User's manual before operation and don't mix different batches of reagents.

1. Sample preparation:

☐ Collect more than 1.2mL sample so that it can repeat test for 3 times;

☐ Sample should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice;

☐ Sample can be stored at 4℃ for 4 days, or stored at -20℃ for 3 months, or stored below -70℃ for long-term storage. Sample repeated freezing and thawing should be avoided;

☐ Recover the sample to room temperature, vortex for 5~10s and centrifuge at 3000rpm for 5s.

2. Consumable preparation:

☐ Magnetic separator, 1.5mL EP tube, 0.2mL PCR 8-tube strip.

3. Reagent preparation:

☐ 3.1 Extraction Kit: S10012E-48, 48T/kit, stored at 2~8℃;

S10012E Extraction Solution 1
S10012E Extraction Solution 2
S10012E Extraction Solution 3
S10012E Extraction Solution 4
Elution Buffer

☐ 3.2 Amplification Kit: S3118E-48 HBV Ultra, 48T/kit, stored at -20℃;

HBV PCR Mix	HBV Negative Control
HBV Enzyme Mix	HBV Quantitative Reference A
HBV Internal Control	HBV Quantitative Reference B
HBV Strong Positive Control	HBV Quantitative Reference C
HBV Weak Positive Control	HBV Quantitative Reference D

☐ 3.3 Sansure Kits Melting:

□ Take all components from extraction kit and equilibrate to room temperature until no ice inside, then gently shake and reverse them upside down several times to mix throughly, especially the **Extraction Solution 2** (Observe the Extraction Solution 2 tube bottom, make sure no magnetic beads accumulation).

□ Take all components from amplification kit and equilibrate to room temperature until no ice inside, then vortex them for 5~10s and centrifuge at 3000rpm for 5s. After centrifuge, no more reverse.

☐ 3.4 Reagents allocation:

Prepare (n+1) tests, where n is the total sample number including controls and references.

□ Reverse the Extraction Solution 2 tube upside down several times to mix throughly again. Observe the tube bottom, make sure no magnetic beads accumulation.

□ Take an EP tube and prepare **Extraction Solution 2 Mix**: 100μL **Extraction Solution 2** + 1μL **HBV Internal Control** / test for (n+1) tests, when adding the IC into the extraction solution 2, pipette several times to mix;

□ Cap and mark the tube, then vortex for 5~10s and centrifuge at 3000rpm for 5s.

4. Extraction:

☐ Nucleic acid lysis:

□ Take (n) 1.5ml centrifuge tubes, cap and mark them according to the samples;

□ Open the tubes lid, pipette 300μL **Extraction Solution 1** + 400μL **Sample** into each tube;

□ Cap the tubes lid, vortex them for 5~10s and centrifuge at 3000rpm for 5s;

□ Open the tubes lid, pipette 100μL **Extraction Solution 2 Mix** into centrifuge tubes;

□ Cap the tubes lid, vortex them for 5~10s;

□ Place the tubes at room temperature for 10 mins.

☐ Nucleic acid binding to the magnetic beads:

□ Centrifuge the above (n) centrifuge tubes at 3000rpm for 5s;

□ Put the centrifuge tubes onto the magnetic separator for 5 minutes, until the magnetic beads attached to the tube wall;

□ Open the tubes lid, gently aspirate the waste reagents out of the tubes, without touching the magnetic beads.

Slowly aspirate with 1000μL tips;

☐ Nucleic acid purified:

□ Keep the above (n) centrifuge tubes on the separator, open the tubes lid, pipette 600μL **Extraction Solution 3** + 200μL **Extraction Solution 4** into each tube;

□ Cap the tubes lid, take out them from the separator, then vortex for 5~10s and centrifuge at 3000rpm for 5s;

□ Put the tubes onto the magnetic separator again for 3 mins, until the magnetic beads attached to the tube wall and the waste reagents being separated into two layers.

[If not two layers, vortex fully and centrifuge again, then repeat this step.]

□ Open the tubes lid, gently aspirate the waste reagents out of the tubes, without touching the magnetic beads.

□ **Firstly, slowly aspirate with 1000μL tips;**

□ **Secondly, centrifuge at 3000rpm for 5s and back to the separator for 3mins, then aspirate with 200μL and 10μL tips for several times until no liquid inside.**

[The waste liquid will affect the amplification process, so this step is essential!]

☐ Nucleic acid eluted:

□ Take the above (n) tubes out from the magnetic separator;

□ Open the tubes lid, pipette 30μL **Elution Buffer** into each tube to wash down the magnetic beads to the tube bottom, and vortex for 5~10s to mix;

□ Cap the tubes lid and place them at room temperature for 10mins;

□ While waiting, prepare **PCR-Master-Mix**:

□ 27.5μL **HBV PCR Mix** + 2.5μL **HBV Enzyme Mix** / test for (n+1) tests;

□ Cap and mark the tube, vortex for 5~10s and centrifuge at 3000rpm for 5s;

□ Centrifuge the samples at 3000rpm for 5s, put the tubes back to magnetic separator again for 3 minutes, until the magnetic beads attached to the tube wall.

☐ Amplification preparation:

□ Take certain number of PCR 8-tube strips according to the samples' amplification layout;

□ Pipette 30μL **PCR-Master Mix** into to each sample PCR well, mark them according to the samples;

□ Pipette 20μL **samples eluted nucleic acid** into each sample PCR well according to the marks;

□ Cap the PCR tubes lid tightly, vortex for 5~10s and centrifuge at 3000rpm for 5s.

[If no centrifuge for PCR tube, pipette several times when adding the samples.]

[Make sure there are no bubbles in the tubes.]

5. Amplification:

☐ Amplification channels setting:

□ FAM (Quencher: None): HBV-DNA;

□ HEX/VIC (Quencher: None): HBV Internal Control;

□ ROX: passive reference. [Only for instruments with ROX correction system]

☐ Amplification volume: 50μL;

☐ Cycle parameters:

	Steps	Temperature	Time	Cycles
1	Uracil-DNA Glycosylase reaction	50℃	2min	1
2	Uracil-DNA Glycosylase inactivation	94℃	5min	1
3	Denaturation	94℃	15sec	45
	Annealing, extension and fluorescence collection	57℃*	30sec	

*Click on 'signal acquisition' in the 3.2 step.