

EXTRACTION PROTOCOL - Qiagen kit - 96 well plates

Label each sample by row & column

Add tissue or wing punches to each well

Make working solution of 180 μ L buffer ATL + 20 μ L (40 μ L for wing punches) proteinase K per well * # wells

Add solution of ProK / Buffer ATL to wells

Seal collection microtubes (wells) and mix by inversion

Centrifuge up to 3000rpm to get all solution to bottom

place in 56 degree oven for at least 2 hours (overnight for wing punches, and vortex every hour or have on a rocking platform)

increase water bath to 70degrees (put Buffer AE in for later)

shake samples vigorously for 15s, then centrifuge to 3000 rpm

Remove caps and add 410 μ L (451 μ L if used 40 μ L ProK, aka x 2.05 amt vol.) premixed buffer AL-ethanol, re-seal and shake vigorously 15s, then centrifuge to 3000 rpm

***removed (place in 70degree water bath for 10 minutes Add 200 μ L ethanol and vortex)

Place marked DNeasy 96 plates on top of S-Blocks

pipet the (up to) 900 μ L mixture from the collection microtubes into the 96 plate. Seal the 96 plate with AirPore Tape Sheet. Centrifuge for 10 mins at 6000 rpm.

Remove tape. Carefully add 500 μ L Buffer AW1 to each well

Seal each 96 plate with a new AirPore Tape Sheet. Centrifuge 5 mins at 6000 rpm.

Remove the tape. Add 500 μ L Buffer AW2 to each sample. DO NOT SEAL with AirPore Tape Sheet. Centrifuge for 15 mins at 6000 rpm.

Place 96 plate in correct orientation on a new rack of Elution Microtubes RS.

Add 200 μ L Buffer AE (heated to 70 degrees for wing punches) to each sample (For wing punches: second elution using same eluate from first elution) and seal with new AirPore Tape Sheets.

Incubate 1 min at room temperature. Centrifuge 2 mins at 6000 rpm