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Extraction of RNA/DNA from Dried Blood on Filter Papers after Long-Term Storage

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Protocol status: Working
We use this protocol and it's working

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Abstract

Modified protocol from the manufacturer's protocol (Qiagen Quick-Start Protocol AllPrep DNA/RNA Mini Kit)

Cutting the sample

1 Using a sterile punch, cut 10 to 12 3 mm circles of blood from the filter paper (the aim is to have at least the equivalent of 50ul of blood for each collection).

Spanish: Cortamos con un sacabocados estéril entre 10 y 12 círculos de 3 mm de tamaño del papel de filtro con sangre (el objetivo es tener un equivalente mínimo de 50ul de sangre para cada extracción)

Dilute the sample for extraction

2 Add 600 µL of RLT Plus Buffer to the 1.5 mL microtube with the blood sample on filter paper cut into small pieces (in 3 mm circles, equivalent to 50ul of whole blood), spin for 00:00:15 at 10000 rpm. 15s

Spanish: Añadir 600 ul de Buffer RLT Plus al microtubo de 1.5mL con la muestra de sangre en papel de filtro cortada en trozos pequeños (en círculos de 3mm, equivalente a 50ul de sangre total), hacer un "spin" por 15 segundos a 10000 rpm.

3 Put the 1.5 mL microtube in a ThermoMixer at 55°C for 00:30:00 at 2000 rpm. 30m

Spanish: Colocar el microtubo de 1.5 mL en un ThermoMixer a 55°C por 30 minutos a 2000 rpm.

4 Add 65 µL of proteinase K, vortex and spin for 00:00:15 at 10000 rpm. 15s

Spanish: Añadir 65ul de proteinasa K, hacer vortex y un "spin" por 15 segundos a 10000 rpm

5 Put the 1.5 mL microtube in a ThermoMixer at 37°C for at least 02:00:00 (120 minutes) at 2000 rpm. 2h

Spanish: Colocar el microtubo de 1.5 mL en un ThermoMixer a 37°C por al menos 2 horas a 2000 rpm.

6 Centrifuge the 1.5 mL tube with the sample for 00:03:00 at 13500 rpm. 3m

Spanish: centrifugar el tubo de 1.5 mL con la muestra por 3 minutos a 13500 rpm.

Sample separation for DNA/RNA extraction

7 Pipette the supernatant carefully to avoid touching the pellet with the tip (approx. 650 µL). Transfer the supernatant into the DNA column (Allprep DNA) with collecting tube. Close carefully and centrifuge for 00:00:30 at 10000 rpm. 30s

Spanish: Pipetear el sobrenadante con cuidado para evitar tocar el precipitado con el tip (aprox. 650ul). Transferir el sobrenadante dentro de la columna de DNA (Allprep DNA) con tubo colector. Cerrar con cuidado y centrifugar por 30 segundos a 10000 rpm.

8 Put the DNA column in a new collecting tube and reserve it. Recover the centrifuged sample (flow through) from the first collection tube and add the same volume of 70° ethanol (approx. 650 µL), mix well with the pipette.

Spanish: Colocar la columna de DNA en un nuevo tubo colector y reservarla. Recuperar del primer tubo colector el centrifugado de la muestra (flow through) y añadirle el mismo volumen de etanol de 70° (aprox. 650ul), mezclar bien con la pipeta.

RNA extraction

9 Transfer 650 µL of the sample homogenate with ethanol to the RNA column with collecting tube, centrifuge for 00:00:30 at 10000 rpm. Discard the flow through and add the remaining 650ul of the sample homogenate, centrifuge for 30 seconds at 10000 rpm. 30s
NOTE: Keep the collecting tube for the next 4 steps.

Spanish: Transferir 650ul del homogenizado de la muestra con etanol a la columna de RNA con tubo colector, centrifugar por 30 segundos a 10000 rpm. Descartar el flow through y añadir los 650ul restantes del homogenenizado de la muestra, centrifugar por 30 segundos a 10000 rpm.
NOTA: conservar el tubo colector para los siguientes 4 pasos.

10 Add 700 µL of RW1 buffer to the RNA column with collecting tube, centrifuge for 00:00:30 at 12000 rpm. Discard the flow through. 30s

Spanish: Añadir 700ul de buffer RW1 a la columna de RNA con tubo colector, centrifugar por 30 segundos a 12000 rpm. Descartar el flow through.

11 Add 500 µL RPE buffer to the RNA column with collecting tube, centrifuge for 00:00:30 at 12000 rpm. Discard the flow through. 30s

Spanish: Añadir 500ul de buffer RPE a la columna de RNA con tubo colector, centrifugar por 30 segundos a 12000 rpm. Descartar el flow through.

12 Add 500 µL RPE buffer to the RNA column with collecting tube, centrifuge for 00:02:00 at 12000 rpm. Discard the flow through. 2m

Spanish: Añadir 500ul de buffer RPE a la columna de RNA con tubo colector, centrifugar por 2 minutos a 12000 rpm. Descartar el flow through.

13 Centrifuge the RNA column again for 00:01:00 at 13500 rpm for better membrane drying. 1m

Spanish: Centrifugar la columna de RNA nuevamente por 1 minuto a 13500 rpm para un mejor secado de membrana.

14 Put the RNA column into a new 1.5mL tube. Add 30 µL of RNase-free water directly onto the column membrane without touching it. Wait 1 minute to hydrate the column. Centrifuge for 00:01:00 at 12500 rpm. 1m

Spanish: Colocar la columna de RNA en un nuevo tubo de 1.5mL. Añadir 30ul de agua RNase-free directamente sobre la membrana de la columna sin tocarla. Esperar 1 minuto para hidratar la columna. Centrifugar por 1 minuto a 12500 rpm.

15 Add another 30 µL of RNase-free water directly onto the column membrane without touching it. Wait 1 minute to hydrate the column. Centrifuge for 00:01:00 at 12500 rpm. 1m

Spanish: Añadir otros 30ul de agua RNase-free directamente en la membrana de la columna sin tocarla. Esperar 1 minuto para hidratar la columna. Centrifugar por 1 minuto a 12500 rpm.

16 Discard the RNA column, correctly label the 1.5 mL microtube and store the extracted RNA at -20°C for early use.

Spanish: Descartar la columna de RNA, rotular correctamente el microtubo de 1.5 mL y guardar el RNA extraído a -20°C.

DNA extraction 7m 30s

17 To the DNA column reserved in step 7. Add 500 µL of Buffer AW1. Centrifuge for 00:00:30 at 12500 rpm. Discard the flow through. 30s
NOTE: Keep the collecting tube for the next 2 steps.

Spanish: la columna de ADN reservada en el paso 7. Añadirle 500ul de Buffer AW1. Centrifugar por 30 segundos a 12500 rpm. Descartar el flow through.
NOTA: conservar el tubo colector para los siguientes 2 pasos.

18 Add 500 µL of Buffer AW2. Centrifuge for 00:02:00 at 12500 rpm. Discard the flow through. 2m

Spanish: Añadirle 500ul de Buffer AW2. Centrifugar por 2 minutos a 12500 rpm. Descartar el flow through.

19 Centrifuge the DNA column again for 00:01:00 at 13500 rpm for better membrane drying. 1m

Spanish: Centrifugar la columna de DNA nuevamente por 1 minuto a 13500 rpm para un mejor secado de membrana.

20 Put the DNA column into a new 1.5mL tube. Add 50 µL of Buffer EB directly onto the column membrane without touching it. Wait 00:01:00 to hydrate the column. Centrifuge for 00:01:00 at 12500 rpm. 2m

Spanish: Colocar la columna de RNA en un nuevo tubo de 1.5mL. Añadir 50ul de Buffer EB directamente sobre la membrana de la columna sin tocarla. Esperar 1 minuto para hidratar la columna. Centrifugar por 1 minuto a 12500 rpm.

21 Add another 50 µL of Buffer EB directly onto the column membrane without touching it. Wait 00:01:00 to hydrate the column. Centrifuge for 00:01:00 at 12500 rpm. 2m

Spanish: Añadir otros 50ul de Buffer EB directamente en la membrana de la columna sin tocarla. Esperar 1 minuto para hidratar la columna. Centrifugar por 1 minuto a 12500 rpm.

22 Discard the DNA column, correctly label the 1.5 mL microtube and store the extracted DNA at -20°C.

Spanish: Descartar la columna de DNA, rotular correctamente el microtubo de 1.5 mL y guardar el DNA extraído a -20°C.