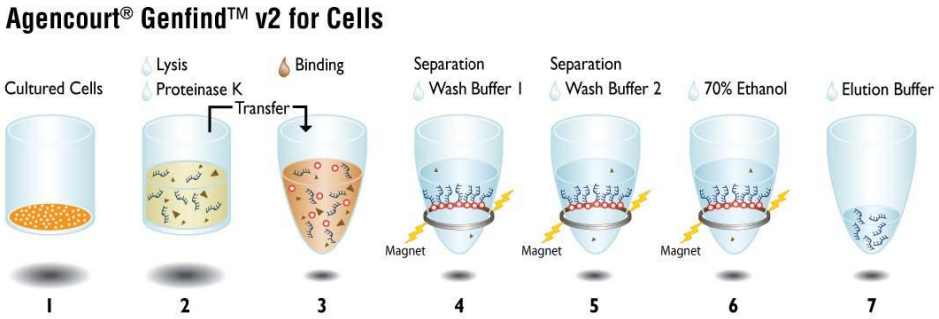
Genfind v2

For extraction of gDNA from cultured mammalian cells

Please reference the current Genfind v2 protocol for product information and a detailed description of use. This protocol is for up to 100.000 cells.

Process Overview:



1. Lyse cultured cells with Lysis Buffer and Proteinase K
2. Bind genomic DNA to paramagnetic beads
3. Separate beads from contaminants
4. Wash the magnetic beads with Wash Buffer 1 to remove protein contaminants
5. Wash the magnetic beads with Wash Buffer 2 to remove salt/small molecule contaminants
6. Wash the magnetic beads with 70% Ethanol to remove residual contaminants
7. Elute DNA from magnetic particles
8. Transfer DNA to new plate

Additional Materials needed:
Culture Plate: Costar 9017: 300ul Flat Bottom Culture Plate: Fisher Scientific #07-200-98
Prep Plate: 96 well 1.2 mL magnet compatible deepwell block: Thermo Fisher #AB-1127
Optional: 100 mg/mL RNase A

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96 Well Purification Procedure (For up to 100,000 cells):

Starting Material: This protocol was designed for purification of cells grown in 96 well culture plates. Following lysis of the cells in this protocol, the samples can be either processed immediately, or the lysate can be stored frozen at -80oC.

1. **Prepare cell culture lysis solution fresh for each extraction according to the recipe below. Use the solution within 45 minutes and discard any unused portion once the extraction is complete.**

   Cell Culture Lysis Solution:
   120 uL Agencourt Genfind v2 Lysis Buffer
   60 uL Reagent Grade Water
   2.7 uL Agencourt Genfind v2 Proteinase K (96 mg/mL)
   Optional: 1ul of RNase A (100mg/mL) if RNA removal is required
   = 182.7ul Cell Culture Lysis Solution per sample (or 183.7ul with RNase)

2. **Remove the culture medium from the cells as completely as possible using a pipette.**

   Tip the plate slightly to one side and place the pipette tip in the corner of the well to avoid aspirating cells from the plate.

3. **Add 182.7ul Cell Culture Lysis Solution to each well. Gently pipette mix 10 times at the bottom of the well to resuspend cells.**

   The lysate may be viscous. Pipetting slowly, or using a larger volume pipette tip for mixing (1mL) may improve mixing.

4. **Incubate the culture plate for 30 min at room temperature to complete the lysis and digestion.**

   Note: Once the 30 minute incubation is complete, the lysate can be frozen at -80°C and extracted at a later time. If freezing samples, seal the plate with an adhesive seal to prevent contamination. Thaw samples at room temperature before resuming the Agencourt Genfind v2 process.

5. **Transfer the entire lysate from the cell culture plate into an AB1127 - 1.2mL 96 well round bottom plate.**

   The lysate may be viscous. Pipetting slowly, or using a larger volume pipette tip for transferring (1mL) may improve the transfer.

6. **IMPORTANT: Vigorously shake the Binding Buffer bottle 20 times to ensure complete resuspension of magnetic particles before using. Add 90 uL Binding Buffer to each sample and gently pipette mix 10 times. Incubate for 5 min.**

   During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is slightly less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

7. **Place the plate on an Agencourt SPRIPlate 96 Ring SuperMagnet for 2 minutes or until the solution clears.**

8. **Aspirate off the supernatant and discard while the plate is situated on the magnet**.

   When aspirating, place the pipette at the center of the well to avoid disturbing the magnetic beads.

9. **Take the plate off the magnet. Add 240 µL of Wash Buffer 1 and pipette tipmix at least 10 times or until the magnetic beads are resuspended from the bottom of the well.**

   Wash Buffer 1 removes protein from the sample. It is normal for a few beads to clump or stick to the bottom of the well. If a white precipitate has formed in the Wash Buffer prior to use, gently shake or stir at room temperature until the solids dissolve. DO NOT HEAT to redissolve.

10. **Place the plate back on the magnet for 1 minute, or until the solution clears.**

11. **Aspirate off the supernatant and discard while the plate is situated on the magnet**.

    When aspirating, place the pipette at the center of the well to avoid disturbing the magnetic beads.

12. **Repeat steps 9-11 using Wash Buffer 2.**

    Wash Buffer 2 removes salts and small contaminant molecules from the sample. The beads should not clump as much as during the Wash Buffer 1 wash.

13. **Repeat steps 10-13 using 70% Ethanol.**

    70% ethanol removes any residual contaminants and traces of Wash Buffers 1 & 2.

14. **Remove as much of the final ethanol wash as possible**.

15. **Add at least 40µL of reagent grade water to each sample to elute.**

    Drying samples is not suggested for this protocol as over-drying the DNA onto the beads makes it difficult to fully elute the samples. If the beads appear very wet, a conservative dry time of 2 minutes at room temperature could be used.

16. Remove the plate from the magnet and resuspend the beads by gently pipette tipmixing 10 times. Incubate the plate for 2 minutes at room temperature, and then pipette tipmix 5 times to complete the elution.

17. Place the plate back on the magnet for 2 minutes, or until the supernatant clears. Transfer the supernatant to a clean plate or clean tubes for storage (-20°C).

If beads are being aspirated during the transfer, dispense the sample back into the well and let the plate sit longer to better compact the bead ring. Leave 5ul of eluant behind to avoid bead carry-over. During the transfer, place the pipette tip in the center of the bead ring and aspirate slowly.