6870

Freezer/Mill®

Tissue Homogenization Cell Lysis

Grinding & Pulverizing

APPLICATION NOTE

RNA Extraction from Cartilage Tissue Using Cryogenic Grinding

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The object of the study was to determine if there is a connection between the seriousness of arthritic diseases, especially of the knee joint and certain genes. To this end the expression profile of various genes in the tissue of healthy and diseased probands was compared. Total RNA was isolated from cartilage tissue and the activity of certain genes was analyzed via PCR or micro array analysis.

Human Cartilage Tissue

Knee cartilage tissue samples were taken from various patients as well as healthy probands, snap-frozen in liquid nitrogen and stored at -80°C. One to two grams of these samples were taken for RNA extraction and added frozen to the grinding container. With the multi-vial adapter option up to four samples can be homogenized simultaneously in the Freezer/Mill® 6850. By utilizing the precool function of the 6850, the samples were held at about -196°C for another two minutes. This assured sufficient cooling and therefore optimum brittleness.

523Grinding Procedure / Homogenization

For technical reasons,—due to the large surface area and the small particle size the cartilage powder showed a tendency to thaw quickly, which should be absolutely avoided (RNA degradation)—the phenol-chloroform reagent (peqGold RNAPure™, peqLAB; Erlangen, Germany) used for further preparation had already been added to the samples (approx. 2 to 2.5ml). After adding the reagent the grinding vials were placed in upright position into liquid nitrogen until the buffer solidified. Then the cartilage samples and the impactor were added to the cooled vial. In order to assure that the impactor not be frozen onto the reagent, the Freezer/Mill was started briefly to check the movement by listening to the sound of the mill.

The grinding was done in three cycles of two minutes each at maximum frequency. Between grinding cycles samples were cooled for two minutes to achieve optimum brittleness again. Thus the whole procedure took 12 minutes. The resulting powder was transferred into Falcon tubes and charged with more peqGOLD reagent (approx. 20ml).

Preparation

The RNA was subsequently purified using a phenol-chloroform extraction method according to the producer's protocol. In order to guarantee optimum RNA clean-up and quality the resulting liquid phase was applied onto RNeasy midi columns.

After photometric examination of the RNA, the samples were analyzed either by quantitative PCR after reverse transcription or alternatively with the Affymetrix GeneChip® system.

The grinding vials were cleaned in several steps. First they were cleaned mechanically with tissue paper and 70% alcohol, then with soap suds. After rinsing with water they were again rinsed with 70% alcohol. This cleaning process should always be carried out promptly as the thawing phenolreagent corrodes the vials (the stainless steel accessories are not affected). Prior to the next use the whole grinding set was autoclaved.

SPEX SamplePrep®

- APPLICATION NOTE SP005: DNA/RNA Extraction
- APPARATUS: **Freezer/Mill®**
- APPLICATION: PCR

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Yield of cartilage RNA was between 2 and 20 µ/g RNA depending on the patient's samples.

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