0805001            DNA Isolation Kit         Zeptometrix      382 €

Below you can read the technical information about the product

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| This Package Insert is provided for product evaluation purposes only and is not intended to be used in place of the Package Insert shipped with the product.  cid:image001.jpg@01C8C498.1456B900  **DNA ISOLATION KIT**  **ATTENTION: RNAse A, Proteinase K, and Desferal  should be removed and stored as is at -20 º C.  All other kit components are stable at 2-4 º C.**  **The development of this product was supported in part by  grants from the National Cancer Institute (R42 CA80451)  and the  New York Center for Biotechnology.**  **FOR RESEARCH USE ONLY. NOT FOR in vitro DIAGNOSTIC USE.**   |  |  | | --- | --- | | **ZMC Catalog # :** | 0805001 |  |  |  |  | | --- | --- | --- | |  |  |  | | **INTENDED USE** |  | The DNA Isolation Kit provides a technique for isolation of genomic DNA from whole blood, cultured cells, and tissues. This procedure provides a rapid method for non-toxic extractions, yielding large quantities of genomic DNA of high purity and minimum oxidative damage. Extracted DNA is suitable for several applications including oxidative DNA damage (1), amplification, restriction enzyme digestion, sequencing, Southern Blot analysis, and other DNA analysis procedures. The DNA yield will vary depending on the type of biological sample and storage conditions.  **For research use only. Not for in vitro diagnostic use.** | |  |  |  | | **PRINCIPLE  OF THE PROCEDURE** |  | Isolation of genomic DNA from cells and tissues, using the Zeptometrix DNA Isolation Kit, involves three major steps. The first step is the isolation of the nuclear fraction. The majority of cellular macromolecules, including RNA, are separated from the nucleus through cell lysis by a non-ionic surfactant. In the second step, RNAse A is used to digest RNA from the nuclear fraction. The sample is then treated with Proteinase K in the presence of SDS, to release the DNA from the nucleus. Proteins in the solution are digested into polypeptides. The third step involves the purification of DNA from the mixture, which is subjected to sodium iodide (NaI) extraction. Polypeptides and other biological molecules remain soluble in the high concentration of sodium iodide. Addition of isopropanol selectively precipitates the DNA. Extraction can be performed by several brief centrifugations in a single Eppendorf microcentrifuge tube.  The addition of Desferal is recommended to reduce artifactual DNA damage during preparation (2,3) .  The DNA Isolation Kit provides reagents necessary to perform 25 to 50 isolations depending on the type and quantity of samples. | |  |  |  | | **PRECAUTIONS** |  | * Please read all instructions carefully prior to performing DNA isolation procedure. * To avoid cross contamination, use separate pipette tips for each specimen. * Universal safety precautions while working with bio-hazardous materials should be adopted (5). * Wear gloves, lab coats and safety glasses at all times. * All contaminated materials should be properly disposed and work surfaces appropriately decontaminated. | |  |  |  | | **REAGENTS** |  | **Materials Supplied:**  •  **Enzyme Diluent,** 10 ml: Contains sterile deionized water •  **10X TE Buffer Solution,** 25 ml: Contains Tris, EDTA •  **Enzyme Reaction Solution,** 60 ml: Contains Tris, SDS •  **Sodium Iodide Solution (NaI),** 60 ml: Contains sodium iodide, EDTA and Tris •  **2X Lysis Solution** , 125 ml: Contains Triton X-100 ® , sucrose, magnesium chloride, and Tris•  **Resuspension Buffer Solution,** 125 ml: Contains sterile deionized water •  **Proteinase K,** (2) 20 mg: Contains Proteinase K •  **RNase A,** (2)10 mg: Contains RNase A •  **Desferal,** 40 mg: Contains Deferoxamine mesylate salt •  **Ribonuclease T1,** 0.150 ml  *Triton X-100® is a registered trademark of Rohm and Haas.*  **Handling and Storage Upon Receipt:**  **Immediately upon arrival, remove RNase A, Proteinase K, and Desferal and place at -20 ° C. Repeated freeze/thaw cycles should be avoided.** The remaining kit reagents should be stored at 2-8 º C. When stored properly, the kit is stable until date indicated on the box label.  **Materials required but not supplied (depending upon sample type):**  **·** 100% Isopropanol **·** 70% Ethanol **·** Phosphate Buffered Saline **·** Deionized Distilled Water (0.45 mm filter sterile) **·** Centrifuge/Microfuge (temperature controlled) **·** Vortex mixer **·** Liquid nitrogen **·** 10-15 ml glass tubes **·** 2 ml Eppendorf tubes **·** Disposable gloves **·** Adjustable pipettes **·** Graduated cylinders and assorted beakers. **·** Heat block, incubator or water bath set at 37ºC **·** Heat block, incubator or water bath set at 50ºC **·** Spectrophotometer **·** Trypsin **·** Mortar and pestle **·** 250 ml centrifuge bottles **·** 15 ml conical tubes | |  |  |  | | **PREPARATION OF REAGENTS** |  | Reconstitute each tube of RNAse A and Proteinase K with 1 ml of Enzyme Diluent. These enzymes can be used directly or divided into single-use aliquots and frozen at -20ºC. Stable up to 6 months when in this state. Avoid repeated freeze/thaw cycles.  **Desferal must be prepared fresh each day. Reconstitute at 1 mg/ml in sterile deionized water. It is added to the Resuspension Buffer Solution, Sodium Iodide, Lysis Solution, and Enzyme Reaction Solution each day at the same concentration in the volumes shown. Discard leftovers.**   |  |  | | --- | --- | | **Volume Of Reagent** | **Volume 1 mg/ml Desferal** | | 10 ml | 657 ul | | 20 ml | 1.314 ml | | 30 ml | 1.97 ml | | 40 ml | 2.627 ml | | 50 ml | 3.284 ml |   The **Enzyme Reaction Solution** and the **Sodium Iodide Solution** may crystallize during storage. If this occurs, warm the solution at 50 º C to solubilize the precipitate. Aliquot the **Sodium Iodide Solution** in small volumes and cap tightly. Protect from light and store at 2-8 º C.  **10X TE Buffer Solution:** Dilute the 10X TE Buffer Solution to 1X by mixing 1 part 10X buffer with 9 parts sterile deionized distilled water. Stable at 2-4 º C.  **Lysis Solution:** Dilute the 2X Lysis Solution to 1X by mixing 1 part Lysis Solution with 1 part sterile deionized water.  **Enzyme Master Solution:** Make fresh each day. For 4 samples, combine 1624.5 µl of Enzyme Reaction Solution, 162 µl of RNAse A, and 13.5 µl of Ribonuclease T1. Mix gently with fingertip and avoid foaming. Scale up as necessary. | |  |  |  | | **SAMPLE PREPARATION** |  | **WHOLE BLOOD:** (100 µl to 1 ml sample)  1. Mix 0.5 ml whole anticoagulated blood with 0.5 ml Lysis Solution. It is important for blood and Lysis Solution to be in equal amounts. 2. Centrifuge at 11000 rpm for 20 seconds at 4 º C, in a temperature controlled centrifuge/microfuge. Pellet is red. 3. Discard supernatant. 4. Add 1ml Lysis Solution to pellet. Resuspend by gentle mixing. Centrifuge for 20 seconds at 11000 rpm. Discard supernatant. 5. Repeat Step 4 until pellet is white. 6. Resuspend pellet in 1 ml Lysis Solution. 7. Proceed to step 3 of DNA Isolation Procedure. **DNA isolated from whole blood using heparin, greater than 20 U/ml may influence the results of the PCR.**  **TISSUE**: (Maximum tissue sample 150 mg)  1. Immerse fresh tissue sample in liquid nitrogen to freeze. Grind sample into powder with a pre-chilled mortar and pestle. 2. Transfer frozen powder to a tube on ice and wash once with PBS. Discard supernatant. 3. Dissolve pellet in 1-2 ml Lysis Solution. 4. Proceed to step 3 of DNA isolation Test Procedure.  **BACTERIA**: (Maximum sample 1010 cells/ml)   1. Add 1 ml cells to 500 ml growth media. Grow culture overnight. 2. Harvest cells in two 250 ml centrifuge bottles. Centrifuge at 5000 rpm for 10 minutes at 4 º C. 3. Discard supernatant. Resuspend pellet by swirling in 100 ml PBS. 4. Harvest cells. Centrifuge at 5000 rpm for 10 min at 4 º C. 5. Resuspend pellet in 50 ml PBS by swirling. 6. Measure A 600 of 1:50 dilution in spectrophotometer for cell count. Each 0.1 OD unit is roughly equivalent to 108 cells/ml. Multiply by 50 for total cell count. 7. Aliquot sample for DNA isolation, with a maximum count of 1010 cells. 8. Centrifuge sample at 11000 rpm for 20 seconds and discard supernatant. 9. Resuspend pellet in 1 ml Lysis Solution by gentle mixing. 10. Proceed with DNA Isolation Procedure, step 3.   Addition of Lysozyme or other appropriate enzymes to the Enzyme Master Solution may be required when working with bacteria or yeast, due to the presence of cell walls. Use at 50 mg/ml, 160 µl per sample.  **ADHERENT CELLS** : (Maximum sample: 37.5 million cells, approximately 2-3 confluent T175 flasks.) ****Cell harvesting by Trypsin****  1.  Remove media from cells. Rinse with PBS. 2.  Add 2-4 ml (depending on size of flask) 0.25% Trypsin-1 mM EDTA. Gently swirl to cover cells. 3.  Carefully discard the solution after 30 seconds. 4.  Cap flask tightly and leave for 1-3 min at 37ºC. 5.  When cells begin to loosen (visually), firmly tap the flask with the palm of the hand to dislodge cells from the bottom of the flask. 6.  Immediately add 5-10 ml of media and rinse flask thoroughly by pipetting buffer over sides of the flask, collecting cells at the bottom of the flask. 7.  Transfer to a sterile conical tube. 8.  Recover cells by centrifuging at 4000 rpm for 6 min at 4ºC. Discard supernatant. 9.  Resuspend pellet gently with 1 ml Lysis Solution. 10.  Proceed to step 3 of DNA Isolation Procedure. | |  |  |  | | **DNA ISOLATION  PROCEDURE** |  | **Harvested mammalian cells:** For oxidative DNA damage applications, all procedures should be carried out at 4 º C except for the enzyme digestions at higher temperatures. Handling of samples should be kept to a minimum and shielded from bright light (1) . Allow the Enzyme Reaction Solution to come to room temperature. The rest of the solutions remain cold  **Step 1: Centrifuge cells at 3000 rpm for 6 min at 4 º C. Decant supernatant.**  **Step 2: Resuspend cell pellet in cold PBS and centrifuge again at 3000 rpm for 6 min at 4 º C. Discard supernatant. Resuspend pellet in 1 ml Lysis Solution.**  **Step 3: Transfer the cell or tissue suspension into a 2 ml Eppendorf tube. Mix gently until completely resuspended.** \*Steps 3-8 should be performed on 12 or less samples at a time, to prevent premature lysis of nuclei due to prolonged exposure to Lysis Solution.  **Step 4: : Centrifuge at 5000 rpm for 2 min. (Do not over spin). Discard supernatant. Large pellets should be split into additional Eppendorf tubes.**  **Step 5: Add 1 ml Lysis Solution. Mix gently to resuspend. Repeat centrifugation step. Remove supernatant completely and discard.** \*Tissues with small nuclei or tough membranes or multiple cell types may need an extra lysis step with or without homogenization. Examples are muscle tissue and zebrafish.  **Step 6: Add 400 µl Enzyme Master Solution. Resuspend gently by flicking tubes. DO NOT VORTEX.  Step 7: Incubate cells or bacteria for 1 hr at 37 º C. Most tissues require 2 hrs. May incubate last 15 min at 50 º C to facilitate digestion.**  **Step 8: Add 20 µl Proteinase K (20 mg/ml stock solution) to each tube and mix by** **inverting 5 times or gentle pipetting up and down ensuring that the pellet is loose and in suspension. DO NOT VORTEX.**  **Step 9: Incubate for 1 hr at 50-60ºC** (4) . **The efficiency of the digestion can be** **monitored visually by clearing of the solution. The incubation time depends on the quantity and quality of sample used. An overnight incubation might be necessary for some tissues. Gentle mixing aids the digestion process.** \*For whole blood samples, the incubation step with Proteinase K differs for different animals: 1hr-human, 2hr- horse, 4hr-cow. \*Longer incubations with Proteinase K may be required for tissue samples with small nuclei or tough membranes, from 3 hrs to overnight depending on when all tissue particulates dissolve. Examples are rat liver, human ovarian tumor cells, muscle cells, and zebrafish. **Centrifuge tubes at 11000 rpm for 5 min to remove undigested material. Transfer the supernates to fresh tubes. If excessive material remains, more Proteinase K can be added to pellets. Repeat incubation and centrifugation.**  **Step10: Add 0.6 ml NaI Solution to each supernate. Mix 3 times by inversion. Once NaI has been added to all samples, mix tubes gently by inverting 60 times or on a gentle rocker. A white precipitate should form.**  **Step11: Fill tubes with 100% Isopropanol and invert until DNA is visible as a stringy gel- like precipitate.**  **Step12: Centrifuge at 11000 rpm for 5 min at 4 º C to pellet the DNA.**  **Step13: Discard supernatant. Blot tubes well to remove remaining NaI. Use fresh pipettes/tips for each sample to prevent cross contamination. Repeat steps 11-13.**  **Step14: Add 1.5 ml 70% Ethanol to each sample. Vortex to mix. \*** If intact DNA is required with minimum breakage, the ends of the tubes must be flicked to mix instead of vortexing. **Centrifuge for 5 min at 11000 rpm and discard supernatant. Repeat 4-7 times or until all NaI is removed. Blot tubes well between each wash. The DNA becomes jelly-like.**  **Step15: Add 0.5 ml 1X TE Buffer or Resuspension Buffer to the pellet. Flick end of tube to dissolve. For oxidative DNA damage studies, rapidly transfer sample into a 10-15 ml conical tube. Recombine split pellets. If pellets adhere to Eppendorf tube, add 0.5 ml Resuspension Buffer and collect in the conical tube. Solubilize by vortexing. Adjust volume to 2 ml. Pellet should become transparent as it dissolves.** \*TE Buffer interferes with oxidative DNA damage studies (1) . In these cases isolated DNA can be dissolved in Resuspension Buffer or water. However, it may take longer (sometimes up to 3-4 days) to dissolve the DNA.  **Step16: Refrigerate overnight at 4ºC to completely dissolve.**  **Step17: Calculate the concentration of DNA using OD260/280 ratio.**  **NOTE: If the DNA is to be used in oxidative damage studies, it may be divided into aliquots, lyophilized and stored at -80ºC (1). Isolated DNA is stable for a year if stored appropriately.** | |  |  |  | | **CALCULATIONS AND INTERPRETATION OF RESULTS** |  | **Determine the optical density of the sample at OD260 and OD280. Calculate the ratio of OD260/OD280 to determine DNA purity. The ratio must fall between 1.7 and 2.0. To determine the yield of DNA calculate the mg of DNA according to the following formula: 1.0 OD260 = 50g DNA**  **Recovery and purity of genomic DNA**   |  |  |  | | --- | --- | --- | | **Sample** | **Recovery** | **260/280** | | Cells (37x106 cells)\* | 200-300 g | 1.96 - 2.0 | | Mouse liver tissue (100 mg) | 250-300  g | 1.96 - 2.0 | | E. coli (1x1010 cells) | 400-500 g | 1.96 - 2.0 | | Blood (100 L-1.0 ml)\*\* | 20-70 g | 1.96 - 2.0 |    \*Mammalian cells used: HeLa, Mouse fibroblast and HL60 \*\*Blood preferred : whole blood anti-coagulated with EDTA, 1.0 mg/ml or Heparin, 10 U/ml. | |  |  |  | | **REFERENCES** |  | 1.     Dawidzik, J.B., Patrzyc, H.B., Iijima, H., Budzinski, E.E., Higbee, A.J., Cheng, H-C, and Box, H.C. (2003) DNA damage measured by liquid chromatography-mass sepctrometry in mouse fibroblast cells exposed to oxidative stress. Biochim Biophys Acta 2003 May 2;1621(2):211-7. 2.     Pouget, J.P., Douki, T., Richard, M.J., Cadet, J. (2000) DNA damage induced in cells by gamma and UVA radiation as measured by HPLC/GC-MS and HPLC-EC and Comet assay. *Chem Res Toxicol* 13, 541 3.     Helbock, H.J., Beckman, K.B., Shigenaga, M.K., Walter, P.B., Woodall A.A., Yeo, H.C., Ames, B.N. (1998) DNA Oxidation Matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc. Natl. Acad. Sci*. 95, 288. 4     Wang,L., Hirayasu,K., Ishizawa,M., and Kobayashi, Y. (1994) Purification of genomic DNA from Human whole blood by Isopropanol-fractionation with concentrated NaI and SDS. *Nucleic Acids Research*. 22, 1174-75 5     MMWR, June 24, (1988), Vol 37, pp. 377-382, 387-388 | |  |  |  | | **PROCEDURAL FLOW CHART FOR MAMMALIAN CELLS** |  | **PREPARE REAGENTS**  **PREPARE SAMPLES**  **CENTRIFUGE SAMPLES AT 3000 rpm FOR 6 MIN AT 4ºC**  **RESUSPEND CELL PELLET GENTLY IN PBS SOLUTION**  **CENTRIFUGE AT 3000 rpm FOR 6 MIN AT 4ºC**  **DISCARD SUPERNATANT**  **REPEAT ABOVE WASH STEPS**  **RESUSPEND IN 1 ml LYSIS SOLUTION**  **CENTRIFUGE AT 5000 rpm FOR 2 MIN AT 4ºC**  **DISCARD SUPERNATANT**  **REPEAT ABOVE THREE STEPS**  **ADD 400 l ENZYME MASTER SOLUTION**  **INCUBATE 1 HOUR AT 37ºC**  **ADD 20 l PROTEINASE K AND MIX**  **INCUBATE AT 50-60ºC FOR 1 HR**  **ADD 0.6 ml SODIUM IODIDE SOLUTION, MIX 60X's**  **ADD 100% ISOPROPANOL, MIX 30X's**  **REPEAT ISOPROPANOL STEP**  **CENTRIFUGE AT 14,000 rpm FOR 5 MINUTES AT 4ºC**  **DISCARD SUPERNATANT AND BLOT**  **ADD 1.5 ml 70% ETHANOL**  **CENTRIFUGE AT 14000 rpm FOR 5 MINUTES AT 4ºC**  **DISCARD SUPERNATANT**  **REPEAT ETHANOL WASH 4-7X's, BLOT**  **ADD 0.5 ml 1X TE BUFFER OR RESUSPENSION BUFFER**  **REFRIGERATE OVERNIGHT TO COMPLETELY DISSOLVE**  **CALCULATE DNA CONCENTRATION USING OD 260/280 RATIO** | |

\* *normal price: 385€, ref : 04-RHUGM-CSF-300µg*

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