Dublin Academic Medical Centre

Standard Operating Procedure
Dublin Academic Medical Centre
UCD Clinical Research Centre

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SOP Title **Biospecimen Storage**

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Purpose

To outline the correct procedures for storing biospecimens.

Specific procedure

1. Staff trained in the receipt of samples shall inspect the sample upon arrival, verifying that the correct commodity was received and that the information on the sample is correct.

2. If the sample container integrity is compromised, the proper amount of sample is not present, or the sample containers are not adequate, document this will be documented at the bottom of the SIF. Documenting date and time received on the SIF is also acceptable.

3. When the samples arrive in the laboratory, they must be registered as records, using the specific computer program for collecting material purpose, labelled with a barcode and collected in racks.

4. All refrigerators and freezers used for samples shall have controlled access. A logbook for each refrigerator and freezer shall be maintained including details, sample traffic and periodic temperature checks. The temperature checks shall be made each working day, or the laboratory may use automatic temperature recording devices. Checks shall be recorded in a logbook.

5. Samples shall be stored in refrigerators and freezers, separate from standards and reagents.

6. Specimens for genomic analysis should be stored at -80oC for a few months.

7. Specimens for DNA, RNA and protein extraction should be divided into three different pieces for the relative analysis. Freezing and thawing should be avoided.

8. Specimens for primary cell culture must be processed immediately in the cell culture laboratory with the correct and proper technique.

9. Blood samples should be centrifuged and the cells separated from the serum into two different containers. They may be stored at -20oC for a few weeks or -80oC for months. Freezing and thawing should be avoided.

10. All human samples should be stored in the specific conditions as suggested by the company guidelines of that specific storing medium.

    The following is s guidline for storage ;
    4oC : DNA in solution
    -20oC : Serum, Urine
    -80oC : RNA, Tissue
    Liquid N2 : Any tissue where viability is to be maintained, PBMCs

Storing and Handling of Nucleic Acid

1. Store genomic DNA at 2 to 8°C. Storing genomic DNA at –15 to –25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at 2 to 8°C or at –15 to – 25°C.

2. Genomic and plasmid DNA can be stored in small aliquots. Repeated use of a single sample may lead to shearing.

3. Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum dried.

4. To help dissolve the DNA, carefully invert the tubes several times after adding buffer and or tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at 2 – 8°C. Minimize vortexing of genomic DNA since this can cause shearing.

5. Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for

pipetting genomic DNA. Regular pipette tips pose no problem for plasmid DNA and other small circular DNA.

6. Extract RNA as quickly as possible after obtaining samples. For the best results, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at –70°C. RNA in inadequately maintained samples can be degraded by intracellular nucleases, especially in tissues that are rich in nucleases (such as spleen and pancreas).

7. RNase inhibitors can be used to protect RNA from degradation both during isolation and purification and also in downstream applications such as reverse transcription into cDNA by RT-PCR, in vitro RNA transcription/translation reactions and RNA-dependent in vitro functional assays. The RNase Inhibitor solution is fully active over a broad temperature range of 25 to 55°C. Even at 60°C some RNase inhibition is still measured. This is advantageous when performing reverse transcription reactions at elevated temperatures to overcome secondary structure in RNA. To keep the inhibitor active, avoid temperatures >60°C or solutions containing strong denaturing agents such as SDS or urea and maintain reducing conditions (1 mM DTT).

8. Store RNA at –70° to –80°C, as aliquots in water. Most RNA is relatively stable at this temperature. Centrifuge the RNA and resuspend in the appropriate RNase-free buffer before use.

9. RNA can be dried briefly at 37°C or in a vacuum oven. When working with RNA, place all samples on ice. For the reasons mentioned above, RNA is very susceptible to degradation when left at room temperature. Dissolve RNA by adding RNase-free buffer or water, then standing the tube on ice for 15 min. Gently tap the tube or use vortexing with caution.

10. Although DNA is relatively stable at elevated temperatures (100°C), most RNA is not (except for short RNA probes, which are stable for 10 min at 100°C). Therefore avoid high temperatures (>65°C) since these affect the integrity of the RNA. Instead, to melt out secondary structures, heat RNA to 65°C for 15 min in the presence of denaturing buffers.

Storing and Handling of Protein

1. The probability of denaturing the protein increases with the number of manipulations required in the experiment. These manipulations include protein sample preparation, purification, and storage.

2. Besides preventing contamination from other laboratory reagents, the use of gloves prevents contamination from proteins commonly found on the skin, particularly proteases, which may degrade the protein sample and destroy activity.

3. Protein solutions should be prepared in high concentration, preferably 1 mg/ml or greater. The high concentration tends to stabilize the protein's native structure as well as inhibiting protein "sticking" to otherwise inert surfaces such as glass and plastic. If high concentrations of the native protein are unrealistic, addition of a second inert protein at high concentration will help prevent losses of protein on inert surfaces. Rinsing with EDTA solution prior to deionized water removes any possibility of contamination by metal ions.

4. Vigorous shaking or stirring (e.g., vortex) can generate shear forces that in certain instances can destroy biological activity. Along with denaturation, protein solutions tend to foam uncontrollably when vortexed. A negative side effect here is oxygenation of the sample buffer, which can result in oxidation of the protein.

5. While working with proteins in the laboratory, they should be kept on ice. Since proteins are generally more stable at colder temperatures, maintenance at low temperatures even for short duration is recommended. Proteins are stored lyophilized, frozen in an appropriate buffer, or refrigerated at 4°C. For short term storage of proteins (hours to days), a standard laboratory refrigerator at 4°C is satisfactory providing the buffer used to solvate the protein provides all the necessary components necessary to stabilize the protein of interest. These components can include reducing agents, hydrophobic additives, and protease inhibitors. Along with the use of gloves mentioned previously,

protease inhibitors prevent denaturation due to contamination from these lytic agents potentially present in the protein source. Additionally, antibacterial agents such as sodium azide can be added to inhibit bacterial growth.

6. Proteins can be stored long term (days to weeks) by quick freezing the sample followed by storage at -20°C. Addition of stabilizers such as glycerol helps prevent damage to the protein during freezing and thawing. Typical concentrations for glycerol are 10% to 50%. Although stable while frozen, repeated thawing and freezing of a sample can lead to degradation and loss of activity. During the freezing process proteins are exposed to extremes of salt concentration and pH. Along with the use of stabilizers such as glycerol, rapid freezing of the protein solution limits the time the protein is exposed to these extreme conditions. The rapid freezing process is typically performed by immersing the protein solution in a dry ice bath containing either acetone or ethanol followed by frozen storage at - 20°C. Along with rapid freezing, the thawing process should also be rapid for the same rationale as when freezing. This can be accomplished by immersion in running lukewarm water. Even when performed rapidly, repeated freezing and thawing of protein samples is considered ill advised. It is advised to divide the original protein sample into several individual aliquots.

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Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.