PRODUCT: **DNAzol® Direct** June 2014
Cat. No: DN 131
Store at room temperature. Keep tightly closed.

PRODUCT DESCRIPTION

DNAzol® Direct is a universal reagent for processing biological samples for direct PCR. No isolation of DNA is required. The DNAzol Direct procedure is simple. Lyse a sample in DNAzol Direct for 15 minutes, add an aliquot of the resulting lysate to a PCR mix, and perform amplification of a selected DNA fragment(s). The standard DNAzol Direct procedure supports PCR amplification of DNA fragments up to 8 kb long.

DNAzol Direct (U.S. patent 7,727,718) composition and procedure are based on the use of an alkaline solution containing polyethylene glycol and other additives. DNAzol Direct quickly and effectively lyses biological samples, releasing DNA into the lysate. The combined effects of the alkaline pH and chaotropic properties of DNAzol Direct sufficiently inactivate PCR inhibitors including proteases and nucleic acid degradation enzymes. After processing a sample in DNAzol Direct, DNA is denatured into a single-stranded form, RNA is hydrolyzed, and proteins are denatured and partially hydrolyzed. Due to its unique composition, the DNAzol Direct lysate does not require neutralization before its use in PCR. The resulting pH of a PCR mix containing less than 10% of the lysate is within the effective range for PCR.

DNAzol Direct is designed to process a wide range of samples including animal, plant, fungi, yeast, bacterial and viral samples. Specific examples are of animal - derived samples: liver, lung, spleen, brain, blood, serum, saliva, hairs and feathers; plant - derived samples: leaves, tissue fragments and seeds; and samples containing yeast, fungi, bacteria and viruses.

STABILITY: DNAzol Direct is stable at room temperature for at least one year after the date of purchase. During storage, keep the bottle tightly closed to prevent neutralization of the reagent by CO_2.

HANDLING PRECAUTIONS: DNAzol Direct is an alkaline solution. Handle with care; avoid contact with skin and use eye protection (shield, safety goggles). In case of contact, wash the skin or flush the eyes with copious amounts of water. For eye splash, seek medical attention.

PROTOCOL

1. Mix 1 - 10 µl of fluid or 1 - 10 mg of solid sample with 0.1 ml of DNAzol Direct.
2. Lyse the sample by incubation in DNAzol Direct for 15 minutes at room temperature.
3. Vortex the lysate and transfer a 2 - 5 ul aliquot directly into 20 -50 ul of PCR mix.

NOTES

1. **The lysate volume should not exceed 10% of the total volume of the PCR mix.** Within this limit, no neutralization of the lysate is necessary for its use in PCR. Using standard PCR conditions, sample lysates in DNAzol Direct support amplification of DNA fragments up to 8 kb long.
2. For optimal amplification, use enough sample lysate to add 10 - 100 ng DNA to a 20 ul PCR mix. Typically, the minimal amount of DNA required for 35 cycles of PCR is 0.1 - 1 ng. However, the DNAzol Direct procedure was used successfully to amplify as little as 7 pg DNA. To calculate the amount of DNA in a sample, assume 1 mg of animal tissue contains 1 - 3 ug DNA and a single mammalian cell contains 6 - 7 pg of DNA.
3. Incubation of samples at 80 - 90 C for 5 - 15 minutes improves release of DNA from samples such as tissue specimens, saliva and dried blood cards. Alternatively, improve release of DNA by incubating samples overnight at room temperature.
4. Samples of solid tissues, buccal swabs and bacterial pellets release abundant amounts of DNA. This excess of DNA and other cellular material can inhibit PCR. In the case of lack of amplification, dilute samples 10 - 50 times with water. For subsequent sample processing, increase the sample-to-reagent ratio.
5. After lysis in DNAzol Direct, samples can be used immediately or stored at room temperature for later use. Whole blood and tissue samples stored in DNAzol Direct for six months at room temperature showed no reduction in the quality and yield of the amplified DNA.
6. DNA fragments amplified by direct PCR with DNAzol Direct lysates can be used for sequencing. In addition to standard PCR, the lysates can be used for multiplex PCR and real-time PCR. Real-time PCR may require dilution of lysates with water.

SPECIFIC APPLICATIONS

Animal tissues. Place 1 mg of tissue in 50 ul of DNAzol Direct. Lyse the sample by incubating for 15 minutes at room temperature or optionally at 5 - 15 min at 80 C. After incubation, vortex the lysate and add 1 - 5 ul aliquot to a PCR mix. Typically, the lysate still contains tissue fragments but sufficient DNA is released to support amplification.

Plant tissues. Place 5 - 25 mg of plant tissue in 0.2 ml of DNAzol Direct and lyse it by incubating for 15 min at room temperature or for 5 - 15 minutes at 80 C. For hard plant tissues containing a high amount of cellulose matrix, increase the amount of tissue 2 - 3 times per volume of DNAzol Direct. To increase the yield of released DNA, disperse the tissue with physical force such as chopping with scissors, homogenization or pulverization in liquid nitrogen.

Whole blood, plasma and serum. Mix 5 - 10 ul of fluid sample with 100 ul of DNAzol Direct. Lyse the sample by incubating for 15 minutes at room temperature, vortex the lysate and take a 2 - 5 ul aliquot for PCR template.

Saliva. Mix 10 ul of saliva with 100 ul of DNAzol Direct. Lyse the sample by incubating for 5 - 15 minutes at room temperature, vortex the lysate and take a 2 - 5 ul aliquot for PCR template. Lysing the sample at 80 C for 10 minutes slightly increases the DNA amplification yield.

Buccal swabs. Lyse the collected material and release DNA by dipping the swab a few times in a tube with 0.3 - 0.5 ml of DNAzol Direct. Store the tube with lysate for 15 minutes at room temperature, vortex for a few seconds, and take a 2 - 5 ul aliquot of the lysate for PCR. Buccal swabs can have a wide variation in the amount of collected cells. In some cases, excess collected cells release large quantities of DNA and other material which may inhibit PCR.

Blood cards. Release DNA from blood cards by incubating the whole blood card or a cut-out fraction of the card in DNAzol Direct for 15 - 60 minutes at room temperature with occasional vortexing. Use an equivalent of 2 -5 ul of blood per 100 ul of DNAzol Direct. Incubating the card in DNAzol Direct at 80 C for 10 minutes increases DNA amplification yield.

Bacteria. Samples containing easy to lyse gram-negative bacteria such as E. coli can be lysed by incubating in DNAzol Direct at room temperature for 15 minutes. Lysis of more resistant gram-positive bacteria requires up to 3 hours of incubation in DNAzol Direct or 10 - 15 minutes at 80 - 90 C.

Proteinase K Digestion.
Digestion. Suspend a sample in a tube with digestion buffer: 50 mM TRIS, 1 mM Ca-Citrate, 10 mM SDS, pH 8.5. Add 27 ul of buffer per 1 - 3 mg of sample. Dried samples may require hydration. Heat dried samples in digestion buffer for 10 minutes at 95 C and then centrifuge briefly to pellet the condensate. Add 3 ul of proteinase K (10 mg/ml water) into the tube. Incubate the digestion for 1 - 3 hours at 60 C until digestion is complete. After digestion, centrifuge the sample at 10,000 g for 5 minutes at room temperature.
DNA Release. Mix 1 part of the supernatant with 10 parts of DNAzol Direct by vortexing. Add 1 - 5 ul of the resulting solution to 50 ul of PCR volume. The DNAzol Direct - tissue digest solution can be used immediately for PCR or stored at room temperature for subsequent use.

Fixed and Paraffin-embedded Tissues.
Paraffin removal. Place 10 - 15 µm of tissue section (1 - 3 paraffin curls; 10 - 30 mg of tissue) in a 1.5 ml tube. Add 1.0 ml of octane and vortex for 10 seconds at maximum speed and incubate at room temperature for 10 minutes with periodic mixing. Add 0.1 ml of methanol to the sample and mix by vortexing. Centrifuge the sample at 7,000 g for 2 minutes and remove the upper layer of octane with a fine tip transfer pipette. After removing the octane, decant the methanol and allow the excess methanol to drain from the tissue pellet. Dry the pellet for 2 - 3 minutes and remove any residual methanol droplets with a micropipette tip.
Proteinase K digestion. Resuspend the tissue sample in 27 ul of digestion buffer and perform the Proteinase K Digestion protocol described directly above. *It is important to note that DNA obtained from fixed tissues often is fragmented significantly and is suitable only for amplification of fragments <500 bp.*

REFERENCES

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3. Finke J, R Fritzen, P Terns, W Lange and G Dolken (1993). An Improved Strategy and a Useful Housekeeping Gene for RNA Analysis from Formalin-fixed, Paraffin-embedded Tissues by PCR. BioTechniques 14, 448.