

## Product Specification Sheet

**Product:** Ampdirect<sup>®</sup> for Human Blood - Shimadzu Direct Amplification Buffer.

**Code:** MBS-109

**Lot #** 11379

**Contents:** one (1) vial 5x Ampdirect<sup>®</sup>-A and one (1) vial 5x Amp Addition-1

**Volume:** 1 mL each

**Physical State:** Liquid (sterile filtered)

**Application(s):** Novel buffer for the direct analysis of Human blood using PCR

**Storage Conditions:** Store kit at -20° C upon arrival. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of receipt.

**Background:** Mammalian body fluids including blood contain many substances that inhibit the activity of enzymes such as *Taq* DNA Polymerase. Therefore it is generally necessary to remove the inhibitory substances and purify DNA before performing DNA analysis on cells present in these samples. Traditional preparation of DNA samples from mammalian cells includes overnight treatment with proteinase K, followed by purification by phenol/chloroform extraction and EtOH precipitation. Recently, many kinds of DNA isolation kits have become commercially available, so DNA isolation has become simpler than before, but is still time-consuming. Further, the recovery and/or purity of the final DNA preparation are not always satisfactory. Finally, the DNA isolation procedure generally increases the likelihood of sample contamination with foreign DNA, including DNA from samples processed earlier. Shimadzu Corporation has developed a novel reagent (Ampdirect<sup>®</sup>) capable of effectively neutralizing the substances in human blood that inhibit DNA amplification. Use of Ampdirect<sup>®</sup> for Human Blood enables direct amplification of DNA from human blood.

### Traditional Method:

Blood sample → <sup>Pretreatment procedure</sup> Lymphocyte separation → <sup>Tens of minutes to half a day</sup> DNA extraction → <sup>DNA amplification</sup> PCR → Detection

### Improved Method by Ampdirect<sup>®</sup>:

Blood sample → PCR → Detection

### Features of Ampdirect:

- DNA present in blood can be amplified directly.
- No DNA extraction is required.
- Only small volumes of blood are necessary for the procedure.
- Risk of cross contamination and/or procedural error is dramatically reduced.
- Three types of anticoagulants (sodium citrate, dipotassium EDTA, and sodium heparinate) are acceptable.
- Blood (citrate or EDTA-treated blood is recommended) stored frozen for long periods can be analyzed.
- Easy PCR method permits mass screening and routine use in a broad field of applications.

**Protocol for Direct PCR of Human Blood<sup>1</sup>:**

Prepare the PCR reaction mixture with 5x Ampdirect<sup>®</sup>-A and 5x Amp Addition-1

↓  
Aliquot 50  $\mu$ L of PCR reaction mixture to each PCR tube

↓  
Add 1.0  $\mu$ L<sup>2</sup> of human blood to each tube<sup>3</sup>

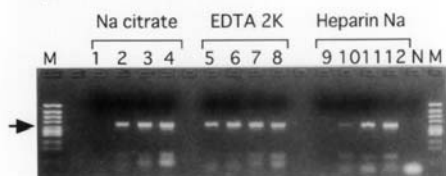
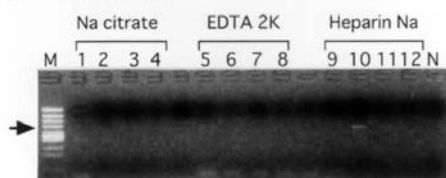
↓  
Overlay 50  $\mu$ L of mineral oil, if needed

↓  
PCR<sup>4,5,6</sup>

**Preparation of the PCR Reaction Mixture:**

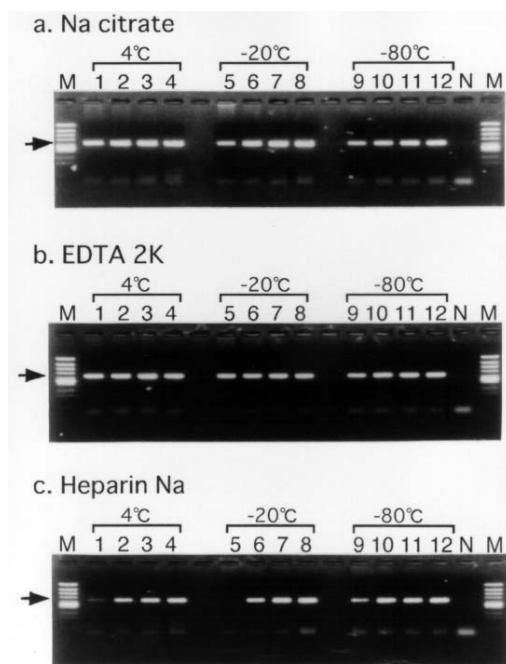
5x Ampdirect <sup>®</sup> -A	10 $\mu$ L
5x Amp Addition-1 (Packaged with Ampdirect <sup>®</sup> )	10 $\mu$ L
dNTP mixture (2.5mM each) <sup>7</sup>	4 $\mu$ L
5'-Primer <sup>7</sup>	0.5 $\mu$ M
3'-Primer <sup>7</sup>	0.5 $\mu$ M
<i>Taq</i> DNA Polymerase (5U/ $\mu$ l) <sup>7,8</sup>	0.25 $\mu$ L
Distilled water <sup>7</sup>	to 50 $\mu$ L

**Experimental Results:** Direct PCR on Blood Treated with Three Different Types of Anticoagulants. Direct PCR on human blood samples treated with three different anticoagulants (sodium citrate, dipotassium EDTA and sodium heparin) was examined. Human blood (0.63-5.00  $\mu$ L) treated with each anticoagulant was added to PCR reaction mixture (final volume 50  $\mu$ L) prepared using Ampdirect<sup>®</sup> for Human Blood. The target sequence ( $\beta$ -globin: 262 bp) was then amplified directly.

**a. Ampdirect mixture****b. Standard mixture**

Lane M contains  $\phi$ X174 RF DNA digested with *HincII*. Lanes 1- 4, 5-6, 9-12 for each coagulant: direct amplification of human blood. Respective volumes of blood within each set were 5.00  $\mu$ L, 2.50  $\mu$ L, 1.25  $\mu$ L and 0.68  $\mu$ L. Lane N contains a negative control. PCR was performed at 94° C for 4.5 min followed by 40 cycles of amplification (94° C for 30 s, 55° C for 1 min, 72° C for 1 min), followed by final extension.

**Experimental Results:** Direct PCR on Blood Stored under Refrigeration or Freezing. Direct PCR was performed on human blood samples stored at 4°, -20° or -80° C for one year. Frozen samples were thawed and refrozen once a month during the storage period. 0.63-5.00 µL of blood was added into reaction mixture (final volume 50 µL) prepared using Ampdirect® for Human Blood. The target sequence (β-globin: 408 bp) was then amplified directly.



Panel a: Sodium citrate-treated blood,  
Panel b: Dipotassium EDTA-treated blood,  
Panel c: Sodium heparin -treated blood.

A: Blood stored at 4° C, B: Blood stored at -20° C  
(thawing and freezing performed once each month), C:  
Blood stored at -80° C (thawing and freezing performed  
once each month).

Lane M contains  $\phi$ X 174 RF DNA digested with *HincII*.  
Lanes 1-4, 5-6, 9-12: Direct amplification of human blood.  
Respective volumes of blood within each set were 5.00 µL,  
2.50 µL, 1.25 µL and 0.68 µL. Lane N contains a  
negative Control. PCR was performed at 94° C for 4.5  
min followed by 40 cycles of amplification (94° C for 30 s,  
55° C for 1 min, 72° C for 1 min), followed by final  
extension.

### Note(s):

The PCR process is covered by world-wide patents owned by Hoffman La Roche.

Ampdirect® and Shimadzu Direct Amplification Buffer were produced by Shimadzu Corporation, Life Science Laboratory (Tsukuba), Analytical Instruments Division, Shimadzu Corporation, 3-17-1 Azuma, Tsukuba, Ibaraki, 305-0031 Japan.

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This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

### Reference(s):

Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, Saha V, Biondi A, Greaves MF. (1999) Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet*. **354**: 1499-1503.

Wiemels JL, Xiao Z, Buffler PA et al. (2002) In utero origin of t(8;21) AML1-ETO translocations in childhood acute myeloid leukemia. *Blood*. May **15**;99(10):3801-3805.

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- <sup>1</sup> We recommend that these procedures be carried out on ice (except when Hot Start PCR is used) to avoid any non-specific reactions and/or reduced sensitivity.
  - <sup>2</sup> We recommend the use of 1.0  $\mu$ L **human** blood in 50  $\mu$ L reaction volume, as excess blood may in some cases reduce efficiency, depending on the individual blood samples and primer sequences used.
  - <sup>3</sup> Any agitation should be strictly avoided.
  - <sup>4</sup> Temperature profile for direct PCR of **human** blood: preheating at 80° for 15 min,<sup>5</sup> then 94° for 4.5 min, followed by 40 cycles<sup>6</sup> of 94° for 30 sec, annealing temperature for 1 min, 72° for 1 min, and finally 72° for 7 min
  - <sup>5</sup> Pre-heating is useful when fresh blood is used.
  - <sup>6</sup> Five more PCR cycles are required than for purified DNA.
  - <sup>7</sup> Not included in Ampdirect<sup>®</sup>.
  - <sup>8</sup> Ampdirect<sup>®</sup> is effective for *Taq* DNA polymerase provided by all manufacturers, with the exception of polymerase activated chemically by higher temperature (e.g. AmpliTaq Gold<sup>®</sup>, Applied Biosystems, Foster, CA, USA and HotStar Taq<sup>™</sup> DNA Polymerase, QIAGEN GmbH, Hilden, Germany). However, *Taq* DNA polymerase plus anti-*Taq* antibody introduced in Ampdirect mixture permits hot start direct PCR.