

Product Specification Sheet

Product: Ampdirect[®] for Mouse Blood - Shimadzu Direct Amplification Buffer.

Code: MBS-110

Lot # 985647

Contents: one (1) vial 5x Ampdirect[®]-B and one (1) vial 5x Amp Addition-2

Volume: 1 mL each

Physical State: Liquid (sterile filtered)

Application(s): Novel buffer for the direct analysis of mouse 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 (Ampirect[®]) capable of effectively neutralizing the substances in mouse blood that inhibit DNA amplification. Use of Ampirect[®] for Mouse Blood enables direct amplification of DNA from mouse blood.

Traditional Method:

Pretreatment procedure Tens of minutes to half a day DNA amplification

Blood sample → Lymphocyte separation → DNA extraction → PCR → Detection

Improved Method by Ampirect[®]:

Blood sample → PCR → Detection

Features of Ampirect:

- 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 routine use (e.g. analysis of transgenic mice).

Protocol for Direct PCR of Mouse Blood¹:

Prepare the PCR reaction mixture with 5x Ampdirect[®]-B and 5x Amp Addition-2

↓
Aliquot 50 μL of PCR reaction mixture to each PCR tube

↓
Add 1.0 μL² of mouse blood to each tube³

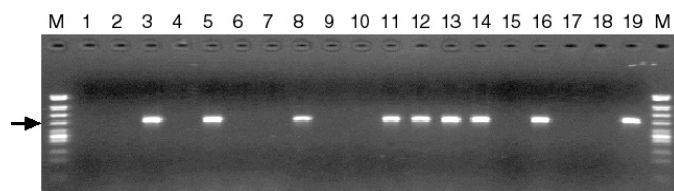
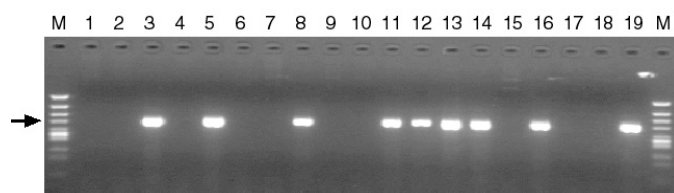
↓
Overlay 50 μL of mineral oil, if needed

↓
PCR^{4,5,6}

Preparation of the PCR Reaction Mixture:

5x Ampdirect [®] -B	10 μL
5x Amp Addition-2 (Packaged with Ampdirect [®])	10 μL
dNTP mixture (2.5mM each) ⁷	4 μL
5'-Primer ⁷	0.5 μM
3'-Primer ⁷	0.5 μM
<i>Taq</i> DNA Polymerase (5U/ μl) ^{7,8}	0.25 μL
Distilled water ⁷	to 50 μL

Experimental Results: Detection of transgene in transgenic mice. Direct PCR detection of the *Ick* promoter-human D4-GDI transgene in 19 transgenic C57BL/6J mice using blood (a), was compared with the results of conventional PCR using purified DNA (b). The specific product of PCR is indicated at 521bp by an arrow.

a. Direct PCR**b. Conventional PCR**

Lane M contains φX174 RF DNA digested with *HincII*. Direct PCR using 1 μL of heparinized blood was carried out in 50 μL of Ampdirect mixture. Conventional PCR using purified DNA obtained from tissue samples of mouse tails was carried out in 50 μL of standard mixture* PCR was performed at 80° for 15 min, then 94° for 4.5 min, followed by 40 cycles of amplification (94° for 30 s, 55° for 1 min, 72° for 1 min), followed by final extension.

* 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM each of dATP, dGTP, dCTP and dTTP, 500 nM each of the specific primers, and 1.25 U of *Taq* DNA polymerase

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.

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. For more information e-mail: nisimura@shimadzu.co.jp Fax:+81-298-51-6682, Tel:+81-298-51-6641

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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.

¹ 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.

² We recommend the use of 1.0 μ L **mouse** blood in 50 μ L reaction volume, as excess blood may in some cases reduce efficiency, depending on the individual blood samples and primer sequences used.

³ Any agitation should be strictly avoided.

⁴ Temperature profile for direct PCR of **mouse** blood: preheating at 80° for 15 min,⁵ then 94° for 4.5 min, followed by 40 cycles⁶ of 94° for 30 sec, annealing temperature for 1 min, 72° for 1 min, and finally 72° for 7 min

⁵ Pre-heating is useful when fresh blood is used.

⁶ Five more PCR cycles are required than for purified DNA.

⁷ Not included in Ampdirect[®].

⁸ Ampdirect[®] is effective for *Taq* DNA polymerase provided by all manufacturers, with the exception of polymerase activated chemically by higher temperature (e.g. AmpliTaq Gold[®], Applied Biosystems, Foster, CA, USA and HotStar Taq[™] DNA Polymerase, QIAGEN GmbH, Hilden, Germany). However, *Taq* DNA polymerase plus anti-*Taq* antibody introduced in Ampdirect mixture permits hot start direct PCR.