Ampdirect® Plus
Procedure

Tissues or fluids from plants or animals contain many substances that can inhibit the activity of enzymes such as *Taq* DNA Polymerase. As a result, it is generally necessary to purify DNA from these samples before performing PCR analysis.

Shimadzu Corporation has for some time sold a PCR buffer series, Ampdirect®, that can neutralize inhibitory substances in biological samples for direct PCR from human and mouse blood and related samples.

Use of the improved Ampdirect® reagent, Ampdirect® Plus, now enables PCR from various kinds of samples containing tissues or fluids from plants or animals.

Product Characteristics

Product Name:	Ampdirect® Plus
Contents:	2x Ampdirect® Plus (containing $MgCl_2$, dNTPs)
Volume:	5 x 1 mL (500 PCR reactions in 20 μL volume, or 200 PCR reactions in 50 μL volume)
Storage:	-20 °C (expiry date as indicated on package label) or 4 °C (1 month) Unsealed package should not be stored on dry ice, to prevent pH drift of the reagent.

Protocol for Sample Preparation

Animal samples such as blood or mucosal cells can be added directly into the PCR reaction mixture.
Solid samples such as plant or animal tissues can be added into the PCR reaction mixture after digestion[1] in the following solution containing SDS and Proteinase K.

Tris.HCl (pH 8.0)	20 mM
EDTA	5 mM
NaCl	400 mM
SDS	0.3 %
Proteinase K	200 μg/ml

[1] Samples should be incubated at 55 °C for 1 hr to overnight.

Preparation of PCR reaction mixtures using our recommended *Taq* DNA Polymerase* (Nova *Taq*™ Hot Start DNA Polymerase (EMD Biosciences, Inc.))

[Reaction volume]	[20 μL]	[50 μL]
2x Ampdirect® Plus	10 μL	25 μL
Nova *Taq*™ Hot Start DNA Polymerase(5 U/μL)	0.1 μL	0.25 μL
10 μM 5'-Primer	1 μL	2.5 μL
10 μM 3'-Primer	1 μL	2.5 μL
Sample	0.5 μL	1 μL
Distilled water	7.4 μL	18.75 μL

* Selection of *Taq* DNA Polymerase besides Nova *Taq*™ Hot Start DNA Polymerase

1 For use of Non-Hot Start *Taq* DNA Polymerase (ordinary r*Taq* DNA Polymerase), we recommend that the PCR reaction mixture be prepared on ice to avoid any non-specific reactions.

2 For use of Hot Start *Taq* DNA Polymerase, we recommend the use of a combination of anti-*Taq* antibody and *Taq* DNA Polymerase (e.g. *TaKaRa Ex Taq*® Hot Start Version (Takara Bio Inc.), Blend Taq™-Plus- (Toyobo Co., LTD.), or Platinum® *Taq* DNA Polymerase (Invitrogen Corp.)).

* Most chemically modified versions of *Taq* DNA Polymerase (e.g. AmpliTaq Gold® (Applied Biosystems) and HotStarTaq® DNA Polymerase (QIAGEN GmbH)) cannot be used.

PCR Condition using Nova *Taq*™ Hot Start DNA Polymerase

95 ℃, 10 min[1]

94 ℃, 30 sec Annealing temp., 1 min 72 ℃, 1 min[2]

30-45 cycles[3]

72 ℃, 7 min

[1] Polymerase activation step for Nova *Taq*™ Hot Start DNA Polymerase.
[2] Longer extension times should be used for amplification of regions larger than 1 kb.
[3] For PCR directly from untreated samples, about five more cycles may be required than for standard PCR from purified DNA.

Note: The PCR process is covered by world-wide patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd.

Ampdirect® Plus is for research use only.

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