Nacalai USA
Innovations for Life Sciences.

Ampdirect® Plus Procedure

Ampdirect® Plus is a novel PCR buffer that effectively neutralizes DNA polymerase inhibitors present in tissues or body fluids from plants or animals, and allows direct PCR amplification from as little as 0.5 ul of sample.

Tissues or fluids from plants or animals contain many substances that can inhibit the activity of Taq DNA Polymerase. As a result, it is generally necessary to purify DNA from these samples before performing PCR analysis. Ampdirect® Plus Kits neutralize charge-bearing inhibitory substance that might bind to DNA polymerase, eliminate all sample purification steps and ensure successful PCR amplification directly from body fluids (i.e. blood, mucosal fluid) and Proteinase K-digested tissue samples.

- **Product Characteristics**

Product Name:	Ampdirect® Plus
Product Number:	07199-20
Contents:	2x Ampdirect® Plus
Volume:	5 x 1 mL (500 PCR reactions in 20 uL volume or 200 PCR reactions in 50 uL 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 ug/ml

[1] Samples should be incubated at 55 °C for 1hr 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 uL]	[50 uL]
2x Ampdirect® Plus	10 uL	25 uL
Nova Taq™ Hot Start DNA Polymerase (5 U/uL)	0.1 uL	0.25 uL
10 uM 5'-Primer	1 uL	2.5 uL
10 uM 3'-Primer	1 uL	2.5 uL
Sample	0.5 uL	1 uL
Distilled water	7.4 uL	18.75 uL

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

1. For use of Non-Hot Start Taq DNA Polymerase (ordinary rTaq 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 HotStartTaq® DNA Polymerase (QIAGEN GmbH)] can not be used.

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

(80 °C, 15 min) [1]
95 °C, 10 min [2]

94 °C, 30 sec Annealing temp., 1min 72 °C, 1 min [3]

30-45 cycles [4]

72 °C, 7 min

1. Pre-heating at 80 °C for 15 min is recommended when fresh blood samples are used.
2. Polymerase activation step for Nova Taq™ Hot Start DNA Polymerase.
3. Longer extension times should be used for amplification of regions larger than 1 kb.
4. 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 produced by Shimazu Biotech in Japan.
Ampdirect® Plus is for research use only.