Terra™ Direct PCR Polymerase Mix Protocol-At-A-Glance

Please read the User Manual before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

1. Terra Direct PCR Polymerase Mix is designed to amplify targets from whole tissues and tissue extracts. Use the sample amount—and/or preparation method—that is appropriate for your sample type.
2. On ice, combine the reagents shown in Table I in a PCR tube. Make sure you use enough of each reagent for all of your reactions plus an additional reaction to accommodate pipetting errors.

Table 1. Recommended Reagent Volumes

Reagent	Volume	Final concentration
2X Terra Direct PCR Buffer (with Mg^{2+}, dNTP)	25 ml	1X
Primer 1	15 pmol	0.3 µM
Primer 2	15 pmol	0.3 µM
Tissue Sample/Extract	≤ 5 µl *	
Terra Direct PCR Polymerase Mix	1 µl	1.25 U
Sterile Water	to 50 µl	
Total volume per reaction	50 µl †	

* When using: 1) crude extracts from various animal tissues, add ≤ 5 µl; 2) blood treated with EDTA or heparin, add ≤ 5 µl; 3) mouse tail, add ≤ 1 mm; 4) mouse ear, add ≤ 1.5 mm2; or 5) plant leaves (e.g., tomato or spinach), add ≤ 1.2 mm diameter disk.

† For 25 µl reactions, be sure to add only half the amount indicated for each reagent.

3. Mix the contents of each tube by tapping the bottom of the tube, then centrifuge briefly.
4. Program your thermal cycler with the following cycling conditions:
   - **3-Step PCR** (for amplification of standard targets < 2 kb):

     98°C 2 min*
     98°C 10 sec
     60°C 15 sec
     68°C 1 min/kb
     ] 30–40 cycles

     * The initial denaturation step must be performed at 98°C for 2 min in order to denature the hot start antibody.

   - **2-Step PCR** (for amplification of targets that are GC-rich or ≥ 2 kb):

     98°C 2 min*
     98°C 10 sec
     68°C 1 min/kb
     ] 30–40 cycles

     * The initial denaturation step must be performed at 98°C for 2 min in order to denature the hot start antibody.
5. Post-PCR Considerations
   - Use TAE running buffer when visualizing your PCR products by agarose gel electrophoresis.
   - Add proteinase K to the gel loading buffer.
     a. Add 5 µl of Proteinase K to 50 µl of 5–6X loading buffer.
     b. Before loading your samples onto a gel, add 15 µl of the loading buffer-proteinase K mixture to the entire 50 µl PCR reaction, or add 1 µl of the loading buffer-proteinase K mixture to 4 µl of the PCR reaction.

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