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Peripheral Blood Sample Processing, RNA Extraction, and qRT-PCR for GRα and GRβ Quantification in NSCLC Patients

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We use this protocol and it's working

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Disclaimer

This protocol is provided for research purposes only. It reflects procedures used in the authors' laboratory and is shared in good faith to facilitate reproducibility and collaboration. Users are responsible for validating the method in their own settings and ensuring that all procedures comply with local institutional guidelines, ethical standards, and safety regulations. The authors and their institutions are not liable for any damage or loss resulting from the use of this protocol.

Abstract

This protocol outlines the step-by-step methodology for peripheral blood collection, buffy coat isolation, RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR) to measure GRα and GRβ expression in non-small cell lung cancer (NSCLC) patients undergoing pemetrexed treatment. It includes detailed instructions for sample processing using Ficoll density gradient centrifugation, RNA quality control, cDNA synthesis conditions, and qRT-PCR execution using SYBR Green chemistry. This standardized procedure enables reproducible quantification of gene expression levels from clinical blood specimens.

Guidelines

This protocol provides standardized procedures for peripheral blood collection, RNA extraction, and qRT-PCR analysis. Users should ensure compliance with institutional biosafety and ethical requirements before performing the protocol.

Safety warnings

Handle human blood samples under biosafety level-2 (BSL-2) conditions and follow appropriate PPE and waste disposal procedures.

Ethics statement

This protocol involves the processing of human peripheral blood samples from patients with non-small cell lung cancer. All procedures were approved by the Ethics Committee of Shiraz University of Medical Sciences (SUMS). The study was conducted in accordance with institutional and international guidelines for human research.
Ethics Approval:
Shiraz University of Medical Sciences Ethics Committee
Approval Code: IR.SUMS.REC.1401.275

Before start

Before starting this protocol, ensure that:
1. Institutional Review Board (IRB) approval for working with human peripheral blood samples has been obtained.
2. All required materials, reagents, and equipment for blood processing, RNA extraction, and qRT-PCR are prepared and calibrated.
3. RNase-free work conditions are established, including cleaned benches, sterilized tools, and use of RNase-free consumables.
4. Personal protective equipment (PPE) such as lab coat, gloves, and face protection is available and used throughout.
5. Sample labeling, documentation templates, and storage conditions (4°C for short-term, –80°C for long-term) are prepared and verified.

Steps

1 Collect 5 mL of peripheral blood in sterile EDTA-K2 tubes during routine follow-up visits.

2 Keep samples at 4°C and process within 2 hours to ensure RNA integrity.

3 Perform leukocyte separation using Ficoll density gradient centrifugation: Layer blood diluted with PBS (1:1) over Ficoll in 15 mL conical tubes and centrifuge at 400 × g for 20 minutes at room temperature with brake off.

4 Collect the buffy coat layer using a sterile pipette and transfer to RNase-free tubes. Store immediately at −80°C until RNA extraction.

5 Extract total RNA using RNeasy Midi Kit (Qiagen, Cat# 74104) according to the manufacturer's instructions. Elute RNA in 100 µL RNase-free water.

6 Assess RNA quantity and purity using NanoDrop ND-1000 spectrophotometer by recording A260, A260/A280, and A260/A230 ratios.

7 Evaluate RNA integrity by 1% agarose gel electrophoresis in 1× TAE buffer stained with SYBR Green II RNA stain. Confirm presence of 28S and 18S bands.

8 Synthesize cDNA from 1 µg of total RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, Cat# K1622) following the thermal profile: 65°C for 5 min, 42°C for 60 min, 70°C for 5 min.

9 Perform qRT-PCR using LightCycler 96 system (Roche) and Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher, Cat# K0221).

10 Include technical duplicates for each sample and non-template control (NTC).

11 Use beta-actin (ACTB) as the housekeeping gene.

12 Calculate relative expression using $2^{-\Delta CT}$ method. Report normalized GRα and GRβ expression levels.