Universal Human Antibody Amplification

Genetic amplification of human antibodies

Catalog Number: NA2204

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

The diversity of antibody variable regions makes cDNA sequencing challenging. Conventional amplification often requires degenerate primers targeting the highly variable 5' end. This kit uses an adapter-based reverse transcription approach designed to capture full-length antibody variable regions efficiently. During cDNA synthesis, an adapter sequence is incorporated to enable downstream amplification. This strategy eliminates the need for highly degenerate primers by pairing a common priming site with chain-specific reverse primers targeting conserved constant regions.

2. Test Principle

This kit provides the specific primers required for the amplification of mouse antibody variable regions (κ, λ, and IgG heavy chains) from hybridoma total RNA. The workflow involves:

Reverse Transcription: RNA is converted to cDNA using primers that bind conserved constant-region sequences. An adapter sequence is introduced during this step to provide a standardized priming site for subsequent amplification.

PCR Amplification: The resulting cDNA is amplified using a forward primer complementary to the adapter and reverse primers specific to the antibody chain type. A nested PCR design improves specificity and product yield.

Sequencing: The resulting ~600-850bp amplicons are purified for Sanger sequencing to determine the variable region DNA sequence.

3. Applications

This enhanced kit is for sequencing the variable regions of human monoclonal IgG antibodies (subclasses IgG1–IgG4) from RNA sources such as recombinant expression cell lines, hybridomas, or single B cells. Using an adapter-based cDNA synthesis approach, the kit now provides complete light chain coverage for both kappa and lambda chains, amplifying IgG heavy chains and kappa light chains to ~750–850 bp, and lambda light chains to ~600–700 bp with newly

integrated human-specific constant region primers. Key limitations include restriction to IgG isotypes, lack of validation for engineered antibodies with altered constant regions or low RNA integrity, and the inability to perform repertoire analysis from polyclonal samples without prior single-cell isolation.

4. Equipment and Reagents Needed (not provided)

- High-quality total RNA from hybridoma cells ($\geq$ 100 ng/μL, A260/A280 $\geq$ 1.8)
- 2X RT Master Mix containing MMLV reverse transcriptase with terminal transferase activity
- 2X High-Fidelity PCR Master Mix
- Nuclease-free tubes and pipette tips
- Thermal cycler
- Agarose gel electrophoresis equipment
- DNA gel extraction/PCR clean-up kit
- Sanger sequencing services

5. Components Provided in This Kit

- 120ul Adapter Oligo (100 reactions) (-80C)
- 120ul RT-Kappa-H Primer (100 reactions) (-20C)
- 120ul RT-Lambda-H Primer (100 reactions) (-20C)
- 120ul RT-Gamma-H Primer (100 reactions) (-20C)
- 120ul Forward Primer (100 reactions) (-20C)
- 120ul PCR-Kappa-H Primer (100 reactions) (-20C)
- 120ul PCR-Lambda-H Primer (100 reactions) (-20C)
- 120ul PCR-Gamma-H Primer (100 reactions) (-20C)

- 1.5ml PCR clean water

6. Reagents Preparation

Master Mix (not Provided)

-Caution this reagent is sensitive to contamination and should only be handled in a clean area away from positive control template.

-Store at -20C. Master Mix is stable when stored at -20C. Freeze and thaw cycles should be minimized to increase shelf life.

-If required aliquots of Master Mix should be made and stored at -20C to minimize freeze thaw and contamination risk.

Primer Mixture

-Store adapter oligo at -80C. All other primers are stable when stored at -20C. Freeze and thaw cycles should be minimized to increase shelf life.

-If required aliquots of Primer should be made and stored at -20C in the dark to minimize freeze thaw and contamination risk.

7. Control Preparation

Negative extraction control (NEC)

-If necessary, prepare one NEC each time extracting RNA from your sample.

-RNase/DNase free water is used in place of sample in the extraction system to create a negative for the DNA isolation method.

- The NEC will serve as a contamination control method for isolation.

No Template control (NTC)

-If necessary, a NTC can made by replacing RNA in the RT reaction with RNase/DNase free water

-The NTC is used to check for contamination during RNA plate set up

8. Assay Set Up

RNA Preparation:

High-quality total RNA must be isolated from hybridoma cells prior to starting the assay. For optimal results, use ≥ 100 ng of RNA per RT reaction with an A260/A280 ratio ≥ 1.8. Assess RNA integrity (e.g., RIN score) if possible.

Experimental Design:

For each antibody sample, three separate RT-PCR workflows must be set up in parallel: one for the kappa (κ) light chain, one for the lambda (λ) light chain, and one for the heavy (γ) chain. This is necessary because the light chain type (κ or λ) is often unknown. Each workflow consists of a reverse transcription (RT) step followed by a nested PCR step.

Controls:

Include the following controls in each run to validate assay performance:

No-Template Control (NTC): Replace the RNA sample with nuclease-free water in the RT reaction. This controls contamination in reagents.

Positive Control Template (PCT): (Optional but recommended) RNA from a known antibody-expressing hybridoma. This verifies the entire process is functioning correctly.

Calculating Reaction Numbers:

Determine the total number of **samples** (including PCT).

For each sample, you will run 3 RT reactions (κ, λ, γ).

For each RT reaction, you will subsequently run 1 corresponding PCR reaction.

Add extra reactions to account for pipetting error (recommended: +10% per step).

Master Mix Preparation:

Prepare master mixes for both the RT and PCR steps to ensure consistency and minimize pipetting error. Always prepare a slight excess (e.g., +10%) to account for pipetting loss.

9. Reverse transcription protocol

1. For each RNA sample prepare three reaction mixtures (one for each chain) according to the table below:

(Include sufficient reactions for positive and negative controls)

RT reaction Setup			
Component	**κ Reaction**	**λ Reaction**	**γ Reaction**
RNA	X (100ng)	X (100ng)	X (100ng)
Adapter oligo (10uM)	1ul	1ul	1ul
RT-Kappa-H Primer (10uM)	1ul	-	-
RT-Lambda-H Primer (10uM)	-	1ul	-
RT-Gamma-H Primer (10uM)	-	-	1ul
2X RT TdT master mix	10ul	10ul	10ul
Nuclease free water	to 20ul	to 20ul	to 20ul

Enter the following amplification program to your PCR machine:

Steps	Time	Temperature
Initial Denaturation	3 Minutes	72C
Reverse transcription	60 Minutes	42C
Enzyme Inactivation	5 Minutes	70C
Hold	indefinite	4C

10. PCR Amplification Protocol

1. each reverse transcription reaction will undergo its respective PCR amplification reaction

according to the chart below.

(Include sufficient reactions for positive and negative controls)

PCR reaction Setup			
Component	**κ Reaction**	**λ Reaction**	**γ Reaction**
DNA from respective RT	2ul	2ul	2ul
Forward Primer (10uM)	1ul	1ul	1ul
PCR-Kappa-H Primer (10uM)	1ul	-	-
PCR-Lambda-H Primer (10uM)	-	1ul	-
PCR-Gamma-H Primer (10uM)	-	-	1ul
2X PCR master mix	10ul	10ul	10ul
Nuclease free water	to 20ul	to 20ul	to 20ul

Enter the following amplification program to your PCR machine:

Steps	Time	Temperature	Cycles
Initial Denaturation	15 seconds	98C	1
Denaturation	15 sec.	98C	10
Annealing	30 sec.	63C to 57.5C (decrease by 0.5°C each cycle)	
Extension	30 sec.	72C	
Denaturation	15 sec.	98C	15
Annealing	30 sec.	56C	
Extension	30 sec.	72C	
Extension	7 minutes	72C	1
Hold	indefinite	4C	1

11. Expected Performance

Gel Electrophoresis Analysis:

Following PCR amplification, analyze 5 µL of each reaction on a 2% agarose gel stained with a DNA-intercalating dye (e.g., SYBR Safe, Ethidium Bromide).

Successful Assay Criteria:

Positive Control (if used): Must show a distinct, single band of the expected size (~600-850 bp) for the appropriate chain(s).

No-Template Control (NTC): Must show no amplification band in any lane (κ, λ, or γ). Any band in the NTC indicates contamination.

Sample Lanes: For a given hybridoma sample, a specific amplification pattern is expected:

Heavy Chain: A single, bright ~750-850bp band should be present in the γ reaction lane.

Light Chain: A single, bright ~600-850bp band should be present in either the κ or λ reaction lane, but typically not both. The other light chain lane may show no band or a very faint, non-specific product.

If these criteria are met, the amplification is considered specific and successful, and the products are suitable for purification and sequencing.

12. Interpreting the test results

Result Pattern (κ / λ / γ lanes)	Interpretation & Next Step
- / - / +	**Amplification Failure (Light Chains).** The heavy chain amplified, but neither light chain did. This suggests poor RNA quality, issues with the light chain RT/PCR steps, or the use of an uncommon light chain constant region. **Action:** Repeat the assay with fresh RNA. If the issue persists, consider verifying RNA integrity or investigating alternative light chain primers.
+ or - / + or - / -	**Amplification Failure (Heavy Chain).** The heavy chain failed to amplify. **Action:** Repeat the assay. Ensure the RNA is of high quality and that the correct master mix

	and thermocycler program were used.
+ / - / +	**Successful Amplification (Kappa Light Chain).** The antibody likely uses a kappa light chain. **Action:** Proceed to purify and sequence both the κ and γ band products.
- / + / +	**Successful Amplification (Lambda Light Chain).** The antibody likely uses a lambda light chain. **Action:** Proceed to purify and sequence both the λ and γ band products.
+ / + / +	**Dual Light Chain Amplification.** Both kappa and lambda chains amplified. This can occur in some hybridomas due to non-productive rearrangements from the fusion partner. **Action:** Purify and sequence all three products. Sequence analysis (looking for in-frame, functional variable regions) or antigen-binding assays on cloned antibodies will identify the correct light chain.
Any Band in NTC Lanes	**Contamination Detected.** The assay is compromised. **Action:** Discard all opened reagents, especially primers and water. Clean workspaces and repeat the assay with fresh aliquots.

13. General Instructions

13.1 Shaking of Reagents

- Shake each reagent gently before using.

13.2 Out of Date Kits

- Don't use kits that have expired.

14. Storage

- Storage condition: -20°C
- Storage period: 12 months

Customer Notes:

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