# (r)Evolution of AAV2 Titration ELISA
## - from monoclonal to recombinant -

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## Abstract & Introduction

PROGEN´s AAV2 Titration ELISA is well known to provide reliable and accurate quantification of AAV2 total capsid titers for many years, based on the sensitivity and specificity of the monoclonal hybridoma antibody A20 for AAV2 intact particles. Murine IgG3 antibodies, like the A20 can be sensitive to storage conditions at low temperatures and show a tendency to oligomerize. Therefore, the production conditions of the A20 antibody need thorough monitoring in order to achieve a high lot-to-lot consistency.
PROGEN now produced a new, recombinant variant of the A20 antibody, called A20R. In contrast to A20, it contains the variable antigen binding region in an IgG1 context instead of the original murine IgG3. Here we demonstrate that A20R shows the same binding specificity to AAV2 compared to A20. Moreover, applying the A20R antibody in the well-established AAV2 ELISA results in the same consistent sensitive and reproducible total capsid titer measurements in comparison to the original A20 antibody.

## Results

### Antibody Stability

#### 1. Gel-filtration of the Recombinant A20 Antibody

A20R was eluted predominantly as a monomeric antibody by gel-filtration.

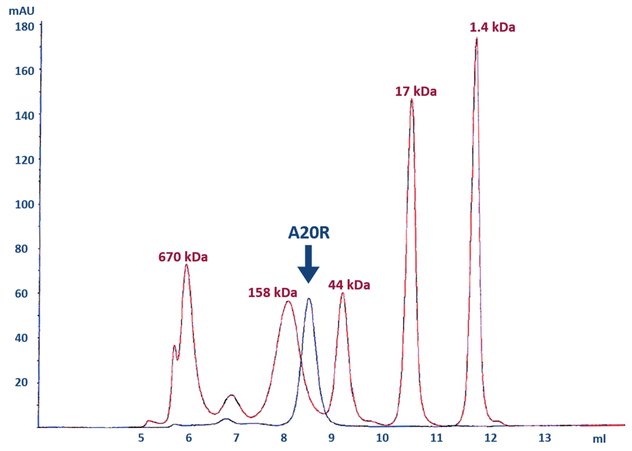
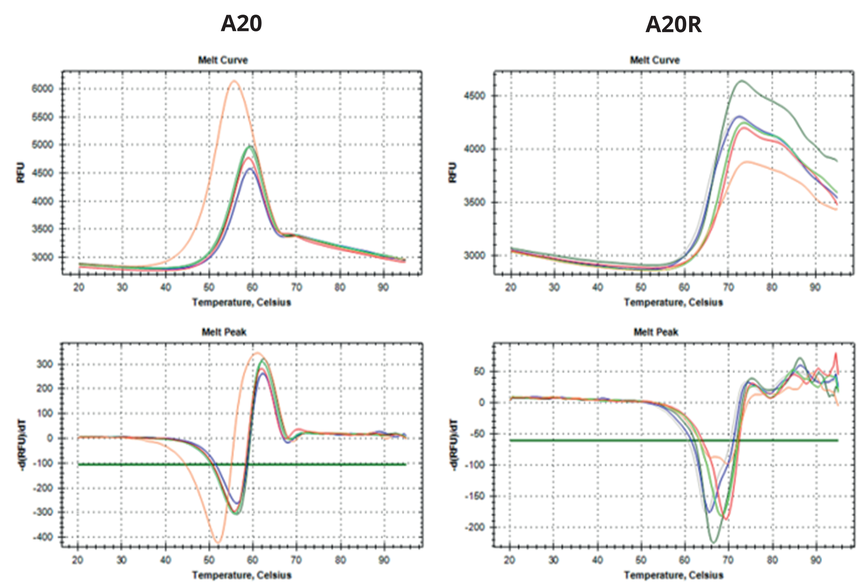



**Figure 1:** Gel filtration of the recombinant A20 antibody (A20R). Red: Elution of known standard proteins. Blue: Elution of the monomeric A20R.

#### 2. Analysis of the Antibody Melting Temperatures by Thermofluor Assay




The A20R showed an increased thermostability.

| Buffer | Melt peak A20 | Melt peak A20R |
|---|---|---|
| pH 5,8 150 mM KCl | 52 °C | 66 °C |
| pH 6,4 150 mM KCl | 55,5 °C | 69,5 °C |
| pH 7,0 150 mM KCl | 55,5 °C | 68,5 °C |
| pH 7,5 150 mM KCl | 56,5 °C | 66,5 °C |
| pH 8,0 150 mM KCl | 56,5 °C | 65,5 °C |
| pH 8,5 150 mM KCl | 56,5 °C | 65 °C |

**Figure 2:** Analysis of the antibody stability of A20 and A20R by thermofluor assay using buffers with different pH (5.8-8.5).

**The A20R elutes as a monomeric antibody from the gel filtration column and shows improved thermal stability.**

### Antibody Binding & Function

#### 3. Binding to AAV2 intact Particles

The A20R recognizes AAV2 intact particles exclusively.

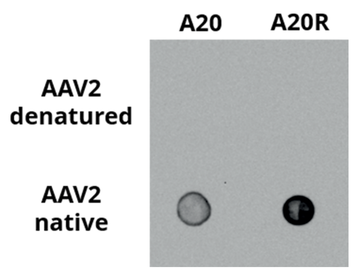



**Figure 3:** Analysis of antibody reactivity with denatured and native AAV2 capsids by dot blot analysis.

#### 4. Antibody Binding Sensitivity

Both antibodies show high sensitivity for AAV2 particles.

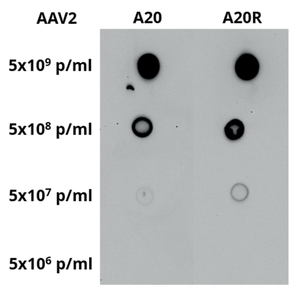



**Figure 4:** Dot blot analysis of binding sensitivity of A20 and A20R by using decreasing AAV2 particle titers.

#### 5. Cross-reactivities with different AAV serotypes

The A20 as well as the A20R recognize only AAV2 and AAV3 intact particles.

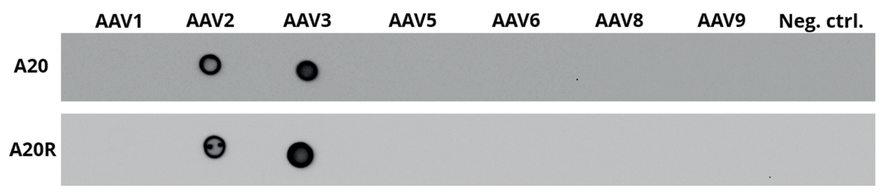



**Figure 5:** Dot blot comparing cross-reactivities of A20 and A20R with different AAV serotypes.

**Note: Differences in the intensity of the spots are not reflecting different affinities. Two different blots were used for the images which result in slightly different signal intensities**

#### 6. Neutralization of AA2 Transduction

The A20R antibody neutralizes transduction of HeLa cells with AAV2 comparable to the A20.

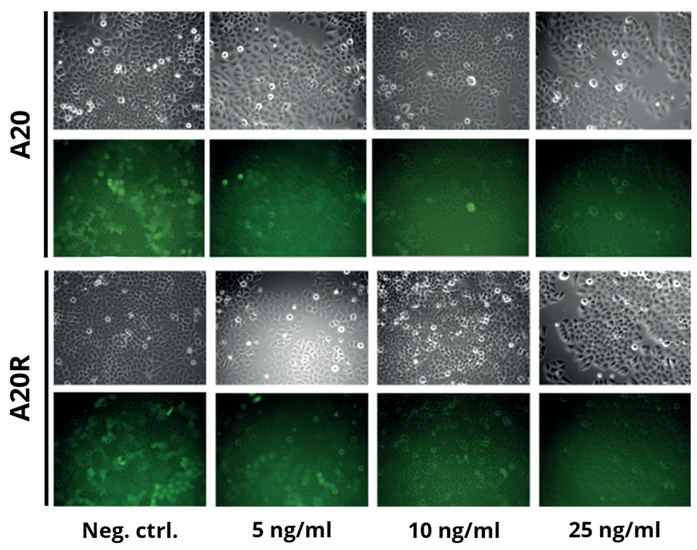



**Figure 6:** Neutralization assay, using different antibody concentrations of A20 and A20R, respectively. AAV2 vectors, conatining a GFP reporter were used for the pre-incubation with the antibodies followed by infection of HeLa cells.

**The A20R shows the same binding specificity for native AAV2 particles as the A20.**

### AAV2 Titration ELISA

#### 7. Comparison of AAV2 Titration ELISA using A20 and A20R

The recombinant A20R can be used in an ELISA as capture and biotinylated detection antibody, showing similar performance to the A20 in terms of sensitivity, stability and reproducibility.

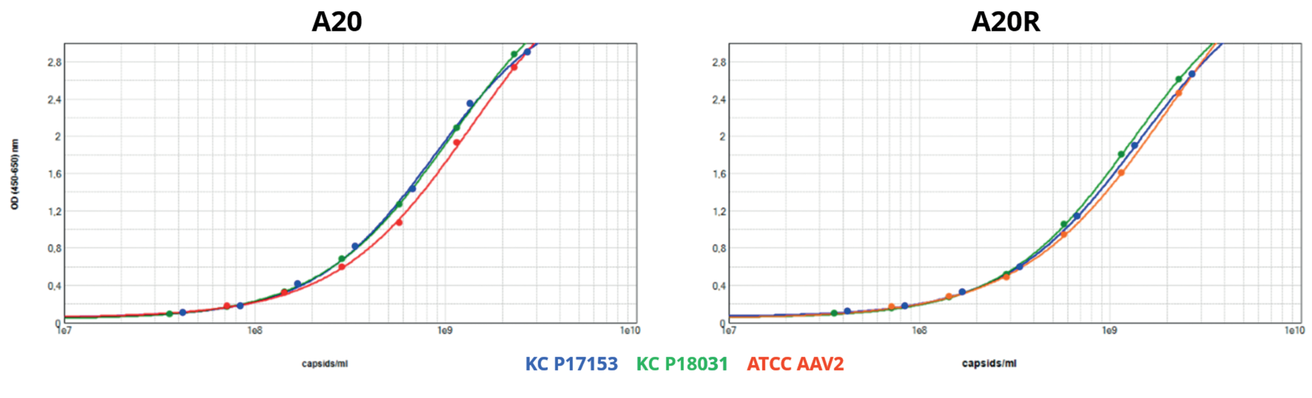



**Figure 7:** Comparison of AAV2 Titration ELISAs using the A20 and A20R antibody, respectively. Blue: Kit Control Lot P17153. Green: Kit Control Lot P18031. Red: ATCC standard material for AAV2.

**A: Intra-Assay Variability**

| Sample No. | P1 | P2 | P3 |
|---|---|---|---|
| tested replicates | n = 24 | n = 24 | n = 24 |
| Average OD | 1.72 | 0.98 | 0.69 |
| Average reading [Capdsids/mL] | 1.1E+9 | 0.53E+09 | 0.36E+09 |
| **CV** | **2.8%** | **3.1%** | **3.0%** |

**B: Inter-Assay Variability**

| Sample No. | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| No. test days | n = 6 | n = 6 | n = 6 | n = 6 |
| Average reading [Capdsids/mL] | 1.14E+9 | 0.76E+10 | 0.55E+09 | 0.38E+09 |
| **CV** | **5.8%** | **5.6%** | **4.8%** | **3.8%** |

**Figure 8: A** Inter-assay variability of the AAV2 Titration ELISA, using the A20R. An established internal control is measured in the same lots on different days. **B** Intra-assay variability of the AAV2 Titration ELISA, using the A20R. Replicates of one sample were measured in the same experiment and plate.

**Both titration ELISAs showed equal recovery of different samples.**

## Conclusion

The new AAV2 antibody, A20R, shows the same binding specificity and sensitivity to AAV2 intact particles but allows easier handling in terms of storage conditions and processing due to improved solubility and thermal stability. In addition, the results confirm the same neutralizing activity for the A20 as well as the recombinant A20R antibody for AAV2 particles encoding a GFP reporter in the cell-based neutralization assay. The newly developed AAV2 Titration ELISA using the recombinant A20R antibody results in the same sample recovery as the AAV2 Titration ELISA using the monoclonal A20 hybridoma antibody. Furthermore, reproducibility of the determined AAV2 total capsid titers has been demonstrated to be comparable to the well-established A20 ELISA. The new AAV2 Titration ELISA using the recombinant A20R is now available (Cat. No. PRAAV2R).

## References

[1]Grimm D, Kern A, Pawlita F, Ferrari F, Samulski R and Kleinschmidt J. Titration of AAV-2 particles via a novel capsid ELISA: packaging of genomes can limit production of recombinant AAV-2. Gene Therapy 6:132-1330 (1999).

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[3]Wobus, C. E. et al. Monoclonal antibodies against the adeno-associated virus type 2 (AAV-2) capsid: epitope mapping and identification of capsid domains involved in AAV-2-cell inraction and neutralization of AAV-2 infection. J. Virol.74, 9281–93 (2000).