



# Calculations for Immunohistochemistry

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# Introduction



- Calculation of a correct dilution is important!
- Often use dilutions with determination of optimal antibody concentration

# New antibody



- Perform doubling dilutions
- Consider detection kit
- Consider secondary antibodies
- Try to use round numbers eg 1/100, 1/500 etc to keep it simple

# A data sheet example



<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
<b>Purity</b>	IgG fraction
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	mAbcam 6147
<b>Isotype</b>	IgG1
<b>Light chain type</b>	kappa

## Applications

Our [Abpromise guarantee](#) covers the use of **ab6147** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★	Use a concentration of 1 - 5 µg/ml.
IHC-P	★★★★☆	Use at an assay dependent concentration.
ELISA	★★★★★	Use a concentration of 0.025 - 1 µg/ml.
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa). Can be blocked with Human Histone H3 (tri methyl K27) peptide ( <a href="#">ab1782</a> ).
Flow Cyt		Use 1-2µg for 10 <sup>6</sup> cells. <a href="#">ab170190</a> - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.



# The common question

- If an antibody is provided at 100ug/ml, and you need 1ug/ml??
- Make sure both numbers are in the same unit – convert both to ug/uL
- $\frac{100}{1000} \times ? = \frac{1}{1000}$



$$\bullet \frac{100}{1000} \times \frac{1}{100} = \frac{1}{1000}$$

- So you need to start with a 1 in 100 dilution to begin with
- One uL (1uL) of concentrated antibody into One hundred uL (100uL) of diluent

# Pipette Caution!



- Beware of the pipette!
- Try to use a minimum of 2uL
- More likely to get reproducible results with volumes more than 1uL.



- So in our example, we are making a 1 in 100 dilution
  - But we aren't using a 1uL volume...
  - So:
    - 2 in 200 dilution
- ➔ 2uL of concentrated antibody in 200uL of diluent.



# How much to make?



- In our example, we made up 200uL.
- An average slide will need 150-200uL when manually pipetted
- Instrumentation will use 100uL per slide

# Doubling dilutions



- Doubling dilutions are a great way of starting an optimisation process (more of that later)
- What are doubling dilutions?



- Otherwise known as serial dilutions
- Step-wise dilutions with each step twice as diluted as the previous one.
- Common way is to start by adding a standard amount of diluent (buffer/PBS or a specific antibody diluent) to three or four tubes.
- Take the same volume from the stock and place into the first tube...





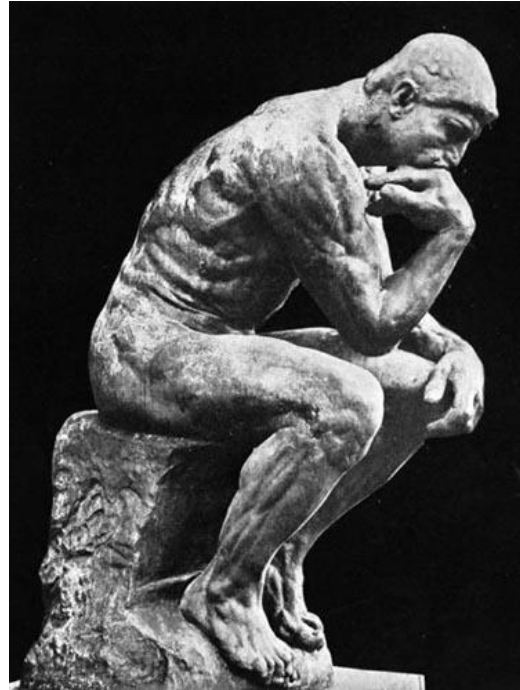
# What if I have need to make a really dilute mix?

- Sometimes antibodies are too concentrated to be used at a low dilution (eg 1/50)
- We can use a stock solution

# Stock solutions



- Say we need to make up a  $1/40,000$  dilution.
- How would we do it?





# One way to make a 1/40,000 dilution

$$\bullet \frac{1}{400} \times \frac{1}{100} = \frac{1}{40000}$$

- So you need to start with a 1 in 400 dilution to begin with
- One uL (1uL) of concentrated antibody into Four hundred uL (400uL) of diluent
- (remembering we try to avoid 1uL so it would be 2uL/800uL).



- Then if we do a 1/100 dilution of the stock, simple maths will show that we now have a 1/40,000 working dilution.
- You would make a 2/200 to avoid a 1ul amount
- You can keep the 1/400 to make up more working dilutions for staining at later date (but beware of expiry timeframes and shelf life of stock solutions!)





# This means you would have 200ul of a 1/40000 solution

- In reality this would stain 2 slides
- Realistically you would either do:
- Stock/diluent
- 10/1000 (to make 1 ml=10 tests)
- 50/5000 (to make 5ml=50 tests)
- 100/10000( to make 10ml=100 tests) etc...



- What other stock solutions could we start with and what dilutions would we make for working dilutions?



- 1/1000 stock with 1/40 working
- 1/4000 stock with 1/10 working
- 1/100 stock with 1/400 working
- The possibilities go on!

# Examples of antibodies



- Renal immunoglobulins IgA, IgG, IgM etc
- Kappa/Lambda
- Kappa (1 in 150000)
- So, Stock solution of 1/150 (or 2 in 300) is made first, from this make a 1 in 1000 (or 2 in 2000).
- It is practical in prep kits to make 5ml (50 tests) so make 5 in 5000ul

# Why do we bother?



- Guidelines on data sheets are not always accurate!
- Serial dilutions can give us a guideline of where to start with our optimisation

# When are dilutions necessary?



- Dilutions and calculations are used for
  - Primary antibodies
  - Secondary antibodies
  - Bulk fluids



## Reaction Buffer Concentrate (10X)

Catalog Number 950-300

### INDICATIONS AND USE

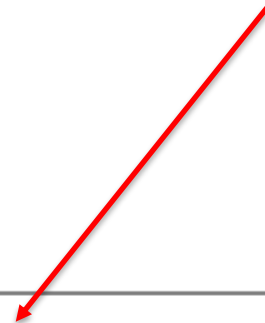
#### Intended Use

This reagent is intended for *in vitro* diagnostic (IVD) use.

Ventana® Medical Systems' (Ventana) Reaction Buffer Concentrate (10X) is a Tris based buffer solution (pH 7.6 ± 0.2) used to rinse slides between staining steps and provide a stable aqueous environment for the immunohistochemistry (IHC) or *in situ* hybridization (ISH) reactions carried out on BenchMark® and BenchMark XT automated slide staining systems.

#### Summary and Explanation

Reaction Buffer Concentrate (10X) is a Tris based buffer solution which must be diluted prior to use. Once diluted, Reaction Buffer (1X) solution is poured into the Reaction Buffer instrument bottle and placed in the appropriate position in the automated fluidics module of the automated slide stainer. The instrument applies Reaction Buffer (1X) solution automatically as required by the procedure being run. For additional information refer to the automated slide staining systems Operator's Manual.





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